1.0 Chapter 1: Introduction

Natural polymers have attracted an increasing attention over the last few decades, mainly due to their abundance, environmental concerns, and anticipated depletion of petroleum resources. This has led to a growing interest in developing chemical and biochemical processes to obtain and modify natural polymers, and to utilize their useful inherent properties for a wide range of applications in different fields (Illum, 1998; Akbuga, 1995).

Among natural polymers, chitosan occupies a special position due to its abundance, versatility, ease of modification and unique properties including biodegradability (Dong *et al*, 2001), biocompatibility (Shigemasa and Minami, 1995; Borchard and Junginger, 2001), non-toxicity (Karlsen and Skaugrud, 1991), anti-bacterial nature (Payne *et al*, 1992) and hydrophilicity (Carunchio *et al*, 1987). This has made chitosan very useful compound in a broad range of applications in medical, pharmaceutical, chemical, agricultural and environmental fields.

To improve chitosan properties and further diversify its applications, various modification strategies have been adopted. This includes: 1) crosslinking (Aly, 1998; Denkbas *et al*, 2000; Yamada *et al*, 2000), 2) graft copolymerization (Yazdani-Pedram *et al*, 2000; Borzacchiello *et al*, 2001; Jenkins and Hudson, 2002; Mahdavinia *et al*, 2004; Huacai *et al*, 2006), 3) complexation (Blair *et al*, 1987; Pedram and Retuert, 1997; Kim *et al*, 2000; Pedram *et al*, 2000; Xie *et al*, 2002; Don *et al*, 2002; Sun *et al*, 2003), 4) chemical modifications (Hall and Yalpani, 1980; Yalpani and Hall, 1984) and 5) blending (Ikejima *et al*, 1999; Ikejima and Inoue, 2000; Cheung *et al*, 2002; Suyatma *et al*, 2004). In particular, modification of chitosan by means of blending is an attractive method that has been

extensively used for providing new desirable characters to chitosan (Taravel and Domard, 1996; Chanachai *et al*, 2000; Smitha *et al*, 2003; Ma *et al*, 2003; Smitha *et al*, 2006). This is mainly due to its simplicity, availability of wide range of synthetic and natural polymers for blending and effectiveness for practical utilization.

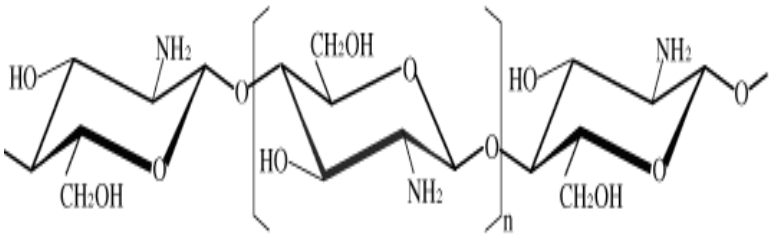
Recently, there has been a growing interest in developing natural based polymeric materials with good film forming capacity for various applications. Among natural polymers, chitosan and its blends can be found in a variey of physical forms including fibers, gel, sponge, beads and nanoparticles in addition to films that can be easily formed. Chitosan films are clear, homogeneous and flexible with good oxygen barrier, mechanical properties and antimicrobial properties (Hoagland and Parris, 1996; Kittur *et al*, 1998). Therefore, they have been applied for a number of applications. For example, chitosan films have been used as a packaging material for the quality preservation of food (Park and Zhao, 2004; Rhim *et al*, 2006; Niamsa and Baimark, 2009). Also, they are used for wound healing in form of bandage (Sathirakul *et al*, 1996), and for producing contact lenses (Olsen *et al*, 1989; Yuan and Wei, 2004).

1.1 Statement of the problem

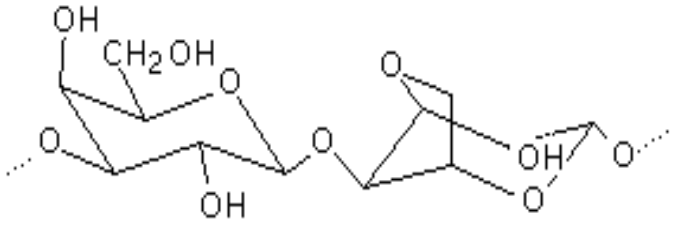
Chitosan is a natural polymer that has been in the center of research attention for the past five decades. This is due to its inherent favorable properties such as biodegradability, biocompatibility, non-toxicity and antimicrobial properties. However, it has some disadvantages such as relatively low degree of swelling, poor tensile strength and low surface wettability of its films, all of which limit the broadening of its scope of applications. To overcome such disadvantages, chitosan is modified by physical blending with natural and/or synthetic polymers.

Blends of chitosan with various natural polymers such as chitosan/cellulose blend and synthetic polymers such as chitosan/polyethylene oxide blend have been reportd in literature in many occations (Hosokawa *et al*, 1990; Khoo *et al*, 2003; Wiles *et al*, 2000). Nonetheless, blends of chitosan with agar (a natural polymer known for high gel forming ability) have not been reported in literature so far. Since chitosan is known for film forming ability caused by rigidity imparted by the presence of hydrogen bonding in its structure, blending it with agar would help to improve its water swelling properties while imparting mechanical strength to the agar. The similarity in the primary structures between agar and chitosan shown in Figure 1.1 suggests a high possibility for formation of a much needed homogeneous blend hydrogel films. The combination of the properties of chitosan including its antibacterial and biocompatibility with gel-forming agar makes the obtained hydrogel blend appealing for some biomedical applications.

Also, blending of chitosan with poly (vinyl alcohol) is expected to form a clear homogeneous hydrogel blend, and therefore, improve some of the properties of chitosan film, such as the mechanical properties due to the specific intermolecular interactions between PVA and chitosan in the blends.



(a)



(b)

$(–CH_2CHOH–)_n$

(c)

Figure 1.1: Chemical structures of (a) chitosan, (b) agar and (c) poly (vinyl alcohol).

1.2 Objectives of the study

The objective of the present study is to investigate the preparation of new chitosan based blends with agar or/and PVA at different proportions and evaluate their properties in the liquid phase and in the solid one represented by blended chitosan films. The objective can be subdivided into the following:

i. To investigate the rheological properties of three types of chitosan based blended solutions: CS/AG, CS/PVA and CS/AG/PVA with respect to apparent viscosity and shear stress as a function of shear rate.

ii. To investigate the effect of variation of the compositions (different proportions) of the three types of blends on the properties of the newly obtained films using various analytical and material research techneques.

1.3 Scope of this study

To achieve the objectives of the present study, the following scope, which is schematized in Figure 1.2, is pursued:

i. Preparation of CS/AG, CS/PVA and CS/AG/PVA blended solutions having different proportions.

ii. Rheological investigation of the three blended solutions represented by apparent viscosity and shear stress as a function of shear rate under different condtions of temperature, concentration, shearing time and time.

iii. Investigation of the chemical composition of the blended films using FTIR.

iv. Investigation of the morphological properties of the blended films using SEM

v. Investigation of the mechanical properties of the blended films using mechanical test.

vi. Investigation of the thermal stability of the blended films using TGA and DSC.

vii. Investigation of the water swelling of the blended films using swelling test.

viii. Invistigation of the surface wettability of the blended films using contact angle analyzer.

1.4 Organisation of the thesis

This thesis is divided into eight chapters: Introduction, Literature Review, Materials and Methods, Results and Discussion (four chapters) and conclusions as follows:

Chapter 1 is a general introduction that gives a brief idea about this work and describes the objectives of the research. In chapter 2, the previous literature related to chitosan and its blends with natural and synthetic polymers is reviewed with special attention giving to the methods of preparation and characterization of chitosan blended films. Chapter 3 deals with materials and procedures for preparing the pure and blended solutions and films of the three polymers used in the current study. It also describes the equipment used to conduct the experimental research. Chapter 4 includes the results of rheological analysis of the three types of polymer blended solutions (CS/AG, CS/PVA and CS/AG/PVA). It also includes the investigation of some parameters affecting the rheology of these mixtures. In chapter 5, blended films of two natural polymers, *i.e.*, CS/AG are characterized by employing FTIR, SEM, thermal studies, mechanical tests, swelling test and contact angle measurements and the obtained results are interpreted and analyzed. In chapter 6, the results of characterization of blends of CS with PVA are presented and discused by using the same techniques mentioned in the previous chapter. Chapter 7 contains the results and discussion of ternary blended films of CS/AG/PVA obtained using similar techniques mentioned in chapter 5. General conclusions and recommendations for future research made based on the present study are provided in chapter 8.

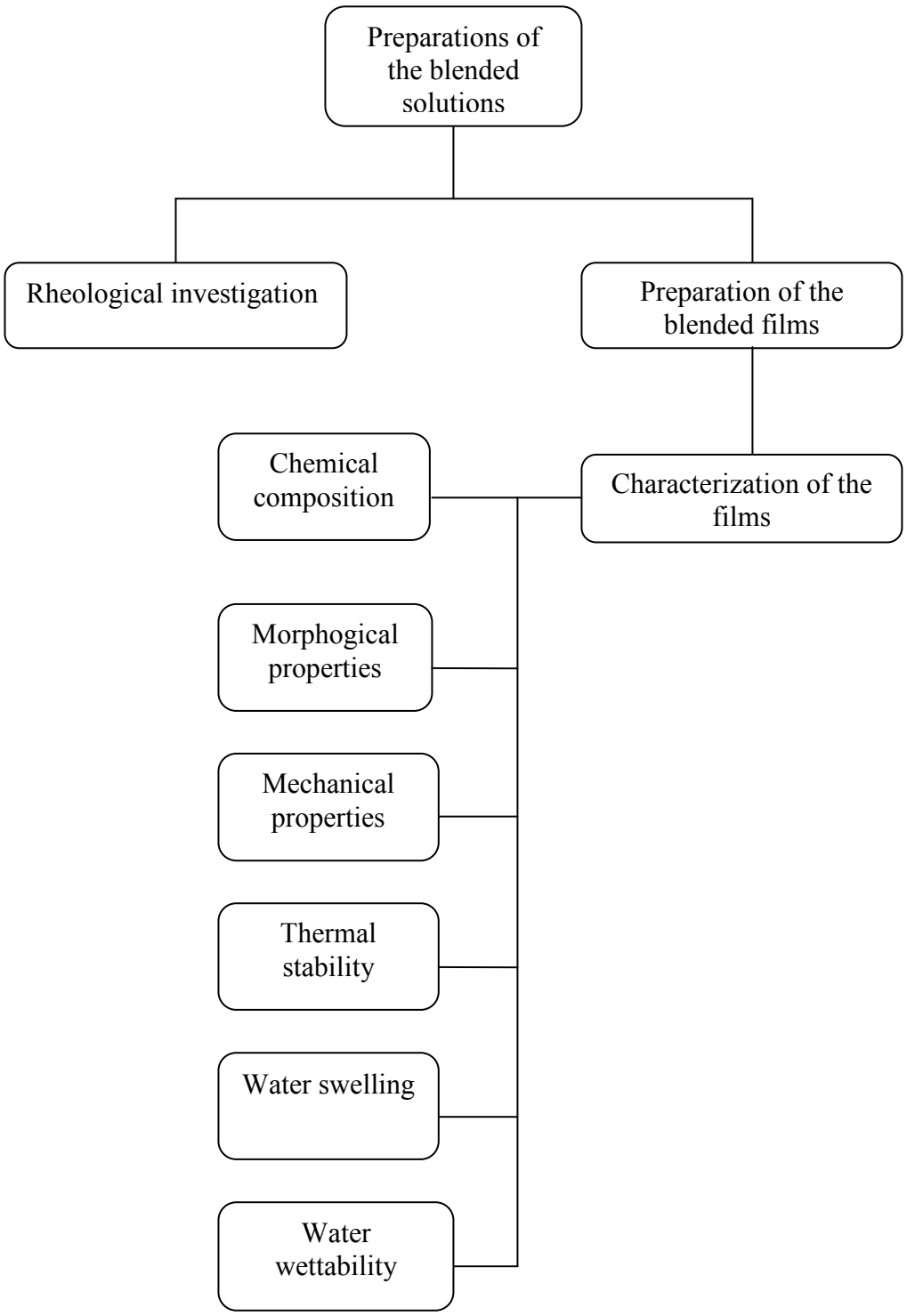


Figure 1.2: Schematic representation of the scope of this study.

2.0 Chapter 2: Literature review

2.1 Introduction

Polysaccharides are a class of natural polymers that have been receiving an increasing attention in various fields of industrial interest including food technology, pharmaceutical and waste water treatment (Wood, 2001). This is because polysaccharides are renewable resources, abundant, nontoxic, inexpensive and biodegradable in addition to their great potential to replace synthetic polymers in many applications (Neto *et al*, 2005). Among polysaccharides, chitosan [Poly (1,4-β-D-glucopyranosamine)], which is usually obtained by alkaline deacetylation of the second most abundant naturally occurring polymer, i.e. chitin, possesses valuable properties (biocompatibility, biodegradability and antibacterial nature) promoting its use in a variety of interesting applications (Suh and Matthew, 2000; Molinaro *et al*, 2002). Furthermore, chitosan has reactive amine side groups, which offer possibilities of modifications, graft reactions and ionic interactions (Yang *et al*, 2004). Therefore, pure and modified chitosan have been evaluated for numerous applications, including medicine, food, cosmetics and wastewater treatment (Cho *et al*, 1999; Pillai and Panchagnula, 2001; Khor and Lim, 2003; Yuan and Wei, 2004; Crini, 2006). Among all modification methods, blending of chitosan with other natural and synthetic polymers has been proposed as convenient and effective means to improve its physical properties to meet the requirements for practical applications. This chapter is devoted for reviewing various aspects of blending of chitosan with natural and synthetic polymers in liquid and solid phases.

2.2 Background and Significance

2.2.1 Occurrence and chemical structure of chitosan

Chitosan, $1 \rightarrow 4$ linked 2-amino, 2–deoxy, β-D-glucan, is the deacetylated derivative of chitin (Muzzarelli, 1973; Bolker, 1974; Dee *et al*, 2001; Singla and Chawla, 2001), the most abundant natural polymer (polysaccharide) on earth after cellulose (No and Meyers, 1989; Kurita, 1998; Bailey *et al*, 1999). The most important sources of chitin today are crustaceans (Sawayanagi *et al*, 1983; Yang and Zull, 1984; Yanga *et al*, 2000; Khan *et al*, 2002), such as shrimps, squids and crabs. Chitin and chitosan are similar to cellulose with respect to their physicochemical properties and their functions due to the similarity in molecular structure. Figure 2.1 shows the molecular structure of chitin, chitosan and cellulose. As it can be seen, the only difference among the three polysaccharides is the acetamide group on the C-2 position of chitin and the amine group in chitosan instead of the hydroxyl group found in cellulose.

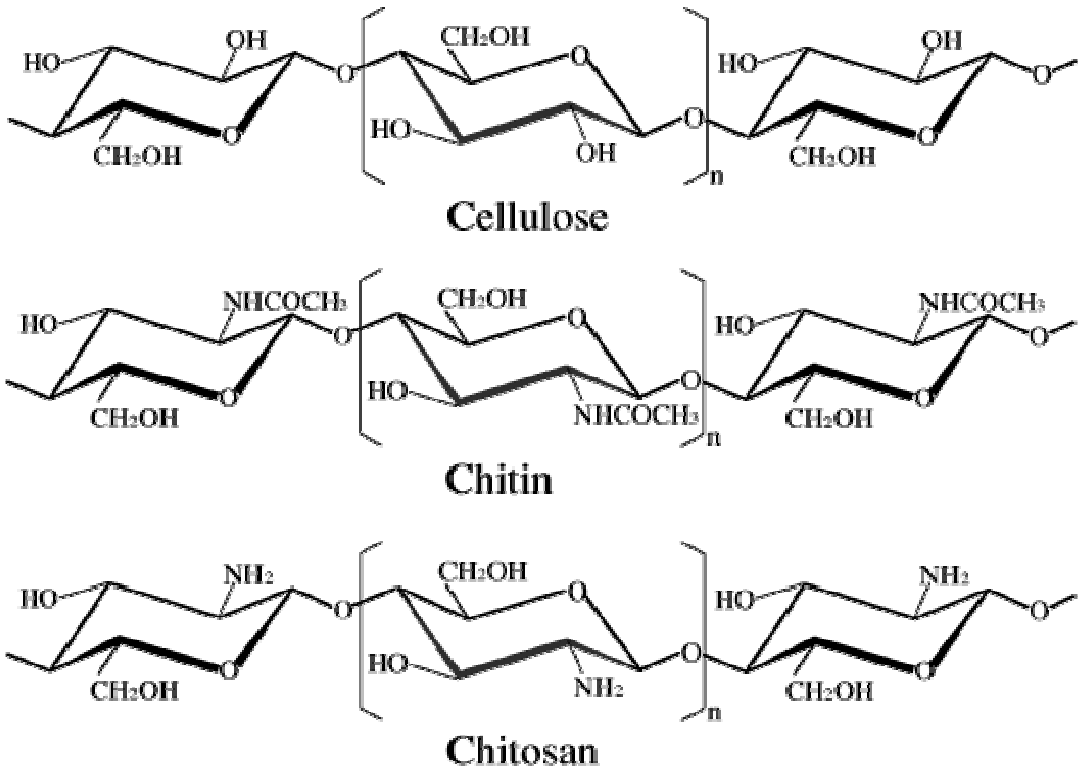


Figure 2.1: Molecular structures of cellulose, chitin and chitosan

2.2.2 Properties of chitin and chitosan

Chitosan has become the subject of research for many research groups due to its unique properties and ease of modification (Mima *et al*, 1983; Seo *et al*, 1991; Mucha, 1997). The physical (Mima *et al*, 1983), chemical (Miyoshi *et al*, 1992) and biological properties (Nishimura *et al*, 1984; Nishimura *et al*, 1985) of chitin and chitosan depend on two parameters: degree of deacetylation (DD) and molecular weight distribution (Rabea *et al*, 2003) which are dictated by the chitin sources and the method of preparation (Brine and Austin, 1981). In fact, the degree of deacetylation of chitosan influences not only its physicochemical characteristics, but also its biodegradability (Hutadilok *et al*, 1995; Nordtveit *et al*, 1996). In addition, the degree of deacetylation is also reported to have an impact on the performance of chitosan in many of its applications (Muzzarelli, 1977; Lower, 1984).

Physical properties

Chitosan exist in a form of white yellowish flakes which can be converted to beads or powder. The degree of deacetylation also plays a vital role on the molecular weight of chitosan. The lower the deacetylation, the higher the molecular weight and higher molecular weight provides higher chemical stability and mechanical strength. The average molecular weight of chitosan is about 1.2×10^5 g mol^{-1} (Nagasawa *et al*, 1971).

Chitin and chitosan are amorphous solids and almost insoluble in water. This is mostly due to the intermolecular hydrogen bonding, which can be formed in the neutral molecules of chitosan. The solubility of chitosan in water depends upon the balance between the electrostatic repulsion resulting from the protonated amine functions and the hydrogen bonding due to the free amino groups (Domard, 1996).

Chitin is insoluble in most organic solvents. It dissolves in corrosive chemicals such as *N*-dimethylacetamide (DMAC) containing lithium chloride. On the contrary, chitosan can be dissolved in aqueous organic acid solutions, such as formic acid and acetic acid at pH below 6, and becomes a cationic polymer due to the protonation of the amino groups available in its molecular structure. However, chitosan hardly dissolves in pure acetic acid. In fact, concentrated acetic acid solutions at high temperatures may cause depolymerization of chitosan. The solubility of chitosan in dilute acids depends on the degree of deacetylation in the polymer chain. Consequently, this property can be used to distinguish between chitin and chitosan (Peter, 1995). The degree of deacetylation is to be at least 85% complete so that the desired solubility can be achieved. Furthermore, the properties of chitosan solutions depend not only on its average degree of deacetylation but also on the distribution of the acetyl groups along the chain (Aiba, 1991; Kubota and Eguchi, 1997), acid concentration and the type of acid (Kurita, 2001).

Chitosan also dissolves in hydrochloric acid under certain conditions. However, it does not dissolve in sulfuric acid because of the formation of insoluble chitosan sulfate (Yamaguchi *et al*, 1978). Aqueous solutions of some acids such as phosphoric, citric and sebacic acids are not good solvents (Gross *et al*, 1983). Moreover, in the presence of a certain amount of acid, chitosan can be dissolved in some solvent mixtures such as water/methanol and water/acetone.

Chemical properties

Similar to most of natural polymers, chitosan has an amphiphilic character, which could influence its physical properties in solutions and solid states. This is attributed to the presence of the hydrophilic amino groups together with the hydrophobic acetyl groups in its

molecular structure. The presence of large number of amino groups also confers chitosan a strong positive charge unlike most polysaccharides. This cationic nature of chitosan makes it modifiable by various means including complexation, grafting, crosslinking and blending (Denuziere *et al*, 1998).

Chitosan is a rigid polymer due to the presence of hydrogen bonding in its molecular structure. Consequently, it can be easily transformed into films with a high mechanical strength. Chitosan is a weak polyelectrolyte which may be regarded as a very poor anion-exchanger. Therefore, it is likely to form films on negatively charged surfaces in addition to the ability to chemically bind with negatively charged fats, cholesterol, proteins and macromolecules (Li *et al*, 1992; Sandford, 1992).

Biological properties

Chitosan is a non-toxic natural product (Arai *et al*, 1968; Hirano *et al*, 1988). Therefore, it can be applied in the food industry for e.g., food for birds and fur-bearing animals. Moreover, chitosan is metabolised by certain human enzymes, especially lysozyme, and is considered as biodegradable (Muzzarelli, 1997; Koga, 1998). Chitosan is also biocompatible and therefore it can play a role in various medical applications such as topical ocular application (Felt *et al*, 1999), implantation (Patashnik *et al*, 1997) or injection (Song *et al*, 2001). Chitosan also has antibacterial (Liu *et al*, 2001) wound-healing effects in human (Ueno *et al*, 2001) and animal (Muzzarelli *et al*, 1988; Okamoto *et al*, 1992; Ueno *et al*, 1999; Mizuno *et al*, 2003) together with hemostatic activities (Malette *et al*, 1983). It also has bioadhesive ability due to its positive charges at physiological pH. (He *et al*, 1998).

2.2.3 Preparation of chitosan and its mixture blends

Preparation of chitosan from raw material

Chitin can be completely acetylated, completely deacetylated and partially deacetylated. In fact, complete deacetylation is rarely achieved. Chitosan is often described in terms of the average molecular weight and the degree of deacetylation (DD). In general, chitin with a degree of deacetylation of 70% or above is considered as chitosan (Li *et al*, 1992).

Chitosan is commercially available. The majority of its commercial samples are available with DD ranges between 70 to 90% and always less than 95% (Guibal, 2004). Tolaimate *et al*. (2003) have reported that chitosan with DD higher than 95% may be obtained via further deacetylation steps. However, this may result in partial depolymerization as well as increase the cost of the preparation. On the other hand, the DD can be lowered by reacetylation (Muzzarelli, 1973).

Generally, chitosan can be produced by the extraction of chitin from the shell of shrimps, lobsters, prawns and crabs, followed by the deacetylation with a strong base heated in special vessels under certain conditions of temperature, atmosphere and time. The obtained chitosan is dissolved in acetic acid and then filtered to remove insoluble material resulting in a clear supernatant solution (Patel *et al*, 2006). This clear solution is neutralized with NaOH solution, which yields a purified sample of chitosan as a white precipitate. In order to obtain medical and pharmaceutical-grade chitosan, further purification might be necessary. Figure 2.2 shows a schematic representation of the processes of preparation of chitosan from raw material.

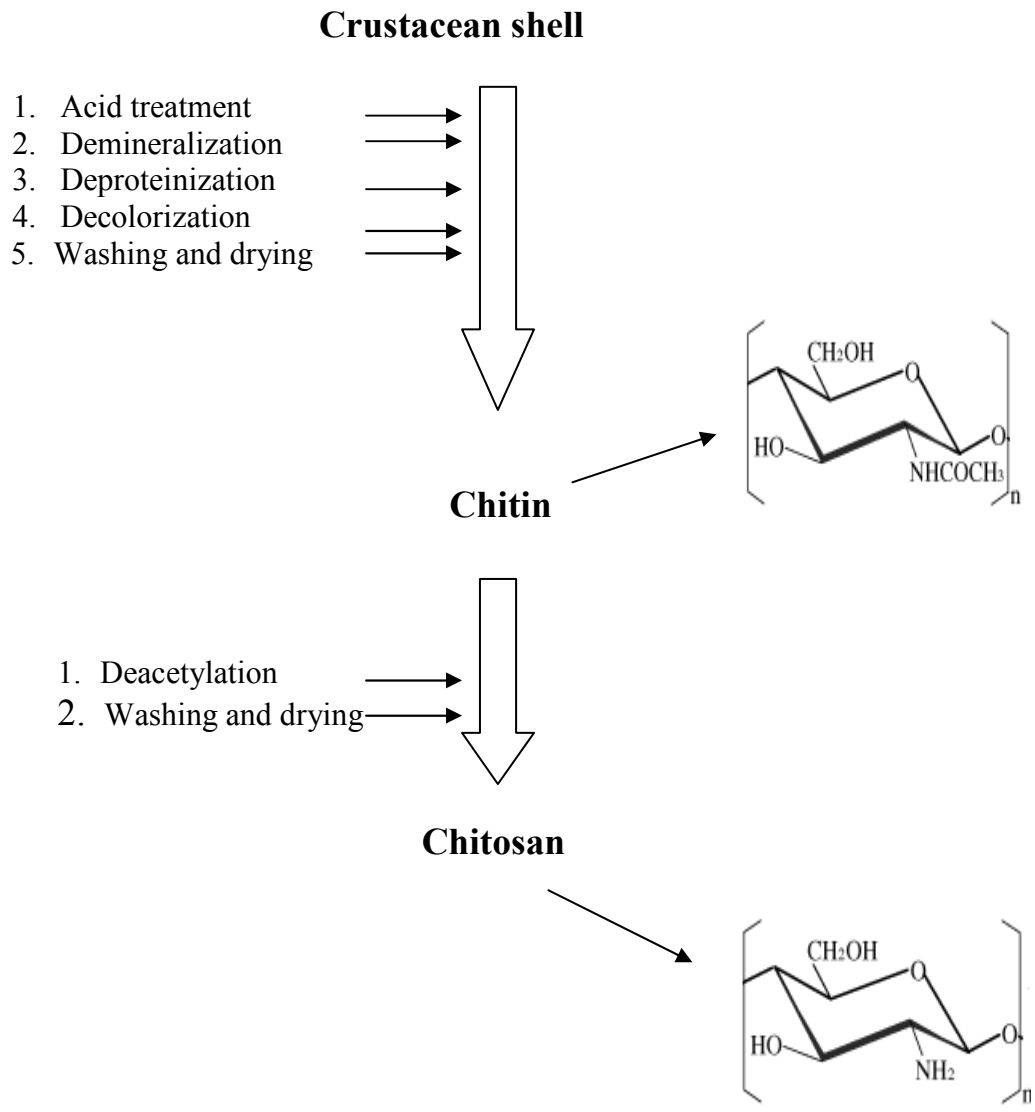


Figure 2.2: Preparation of chitosan (No and Meyers, 1995; No *et al*, 2000).

2.2.4 Some applications of chitosan

In medical and pharmaceutical field, chitosan can be used perfectly for promoting weight loss (Luyen and Rossbach, 1992). This is due to its capability of binding a high amount of fats (about 4 to 5 times its weight) compared to other fibers. In addition, chitosan has no caloric value as it is not digestible, which is a significant property for any weight-loss product (Kanauchi *et al*, 1995). Therefore, it is used as cholesterol reducing agent (Gordon,

1984). Chitosan is also used as a wound healing agent (Lioyd *et al*, 1998; Cho *et al*, 1999; Ueno *et al*, 1999; Ueno *et al*, 2001) in the form of a bandage (Sathirakul *et al*, 1996), dressing burns (Allan *et al*, 1984), drug carriers (Pillai and Panchagnula, 2001; Hejazi and Amiji, 2003; Khor and Lim, 2003), drug delivery system (Sawayanagi *et al*, 1982; Park *et al*, 2001a; Nunthanid *et al*, 2004), preventing heart disease, controlling high blood pressure, preventing constipation, reducing blood levels of uric acid and producing artificial kidneys (Hirano and Noishiki, 1985) due to the high mechanical strength of its membrane. In the eye-wear industry, chitosan has been used to produce contact lenses (Markey *et al*, 1988; Olsen *et al*, 1989; Yuan and Wei, 2004) more biocompatible than those made of synthetic polymers (Allan *et al*, 1984). In cosmetics and personal care (Gross *et al*, 1983), it is used in face, hands and body creams. In addition, it can be applied in bath lotion and hair treatment due to its cationic charge, which makes it interact easily with negatively-charged tissues like skin and hair (Griesbach *et al*, 1999).

In chemical industry, chitosan is also used in the paper industry as a thickener for printing and in coating paper because it is capable of interacting with fibers to form some bonds such as ionic, covalent and Van der Waals forces bonds which enhance paper stability and its resistance to outer influences (Muzzarelli, 1977). In addition, owing to the smoother surface and resistance to moisture of the paper produced with chitosan, chitosan is used in the production of toilet paper, wrapping paper and cardboard. In the photography field, chitosan is used for rapid development of pictures.

In agriculture, chitosan is safely used for controlled agrochemical release, seed coating (Rawls, 1984) and making fertilizer due to its biodegradability and natural origin. In the

recently years, there has been some concerns over its use in biomedical applications (Li *et al*, 1992; Suh and Matthew, 2000; Risbud *et al*, 2002).

In environmental applications, chitosan plays an important role in wastewater treatment and industrial toxic pollution management (Muzzarelli, 1977; Crini, 2006) as it has the ability to adsorb dyes, pesticides, and toxic metals from water and waster water. In addition, fibers of chitosan containing some enzymes are used in filters of gas masks as they detoxify harmful gases (Ember, 1997).

2.2 Rheology of chitosan and its mixtures

2.2.1 Introduction

Rheology is defined as the science of the deformation and flow of matter (Scott-Blair, 1969; Steffe, 1992; Rao, 1999). It investigates the response of materials to applied stress or strain (Herh *et al*, 2000; Hackley and Ferraris, 2001). Rheological properties describe flow characteristics and textural behavior of substances. In fact, the success of a wide range of commercial products and industrial processes depends on meeting specific flow requirements. Rheological behavior can be generally divided into two types (Lee *et al*, 2009): (i) elastic behavior where the material restores its original shape when the external force is removed (ii) viscous or plastic behavior in which any deformation ceases when the applied force is removed, such as ideal Newtonian liquids. The fluids flow behavior is summarized in Figures 2.3 and 2.4. Rheology is an important tool in several fields including food industry, coating, personal care products and cosmetics, detergents, cement and medicine.

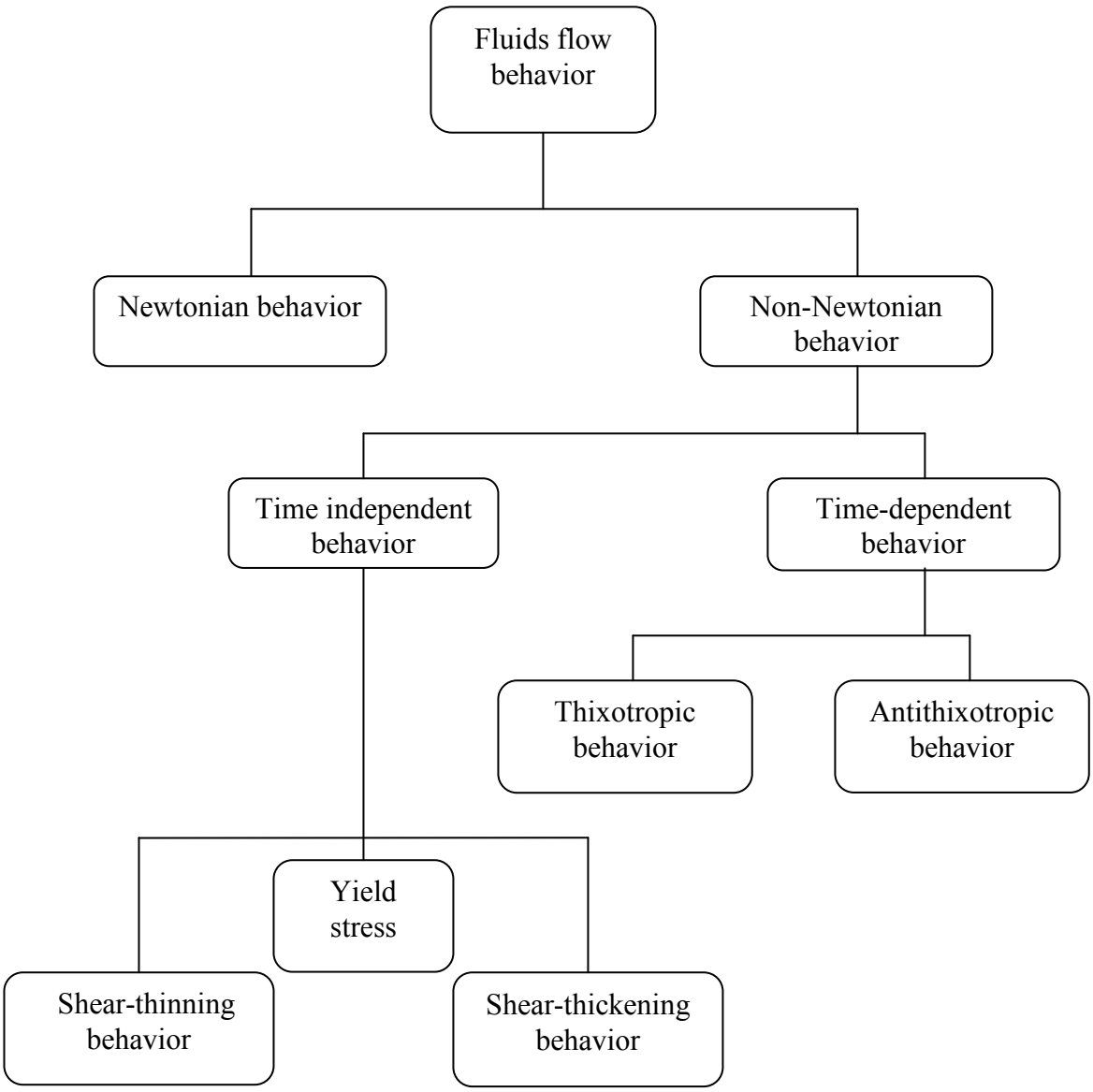


Figure 2.3: Fluids flow behavior.

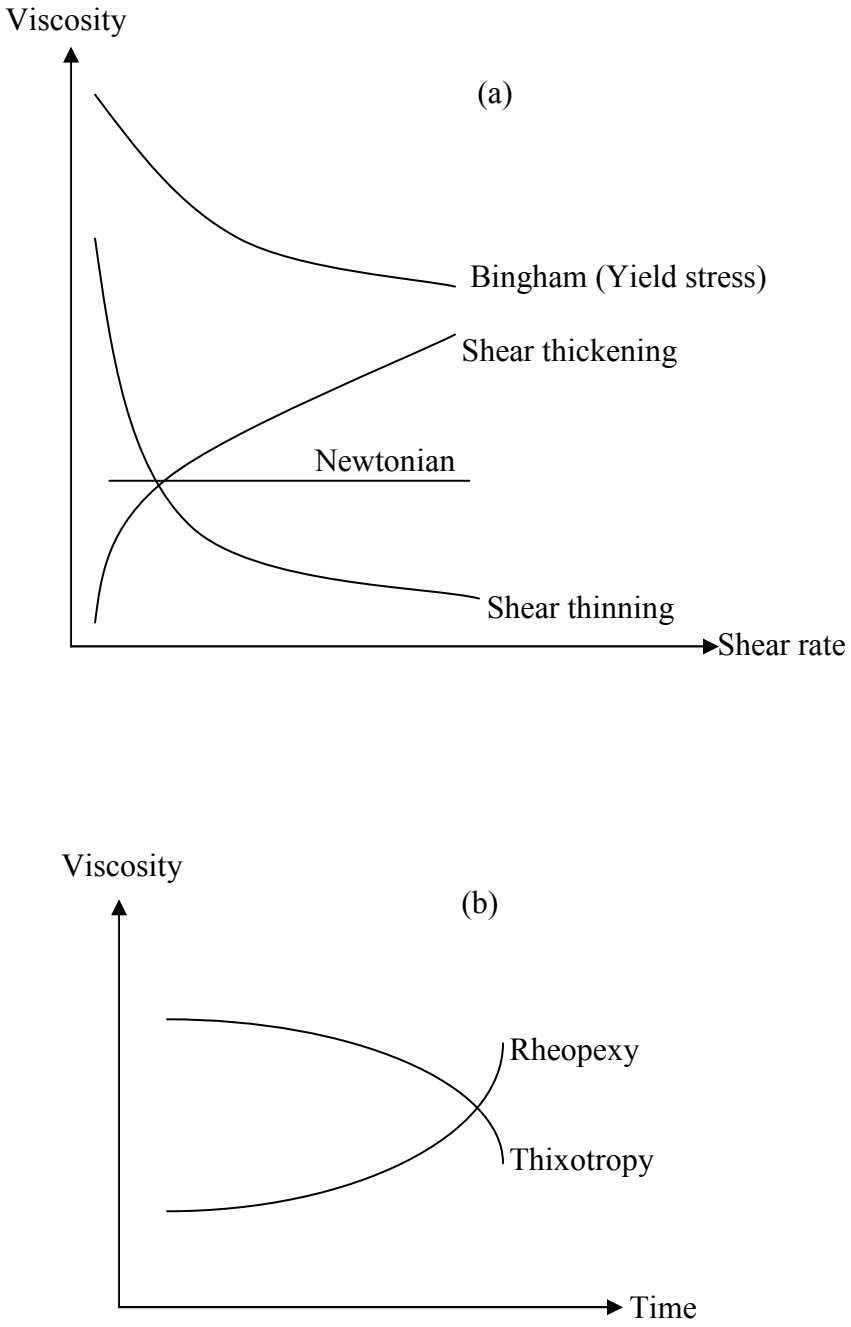


Figure 2.4: The relationship between apparent viscosity and shear rate for (a) time-independent flow fluids and (b) time-dependent flow fluids.

2.2.2 Literature work on rheology of chitosan

Rheology of pure chitosan solutions and gels

The rheological behavior of chitosan solutions has been reported in the literature in various occasions (Berkovich *et al*, 1980; Kienzle-Sterzer *et al*, 1982; Kienzle-Sterzer *et al*, 1985; Wang and Xu, 1994; Mucha, 1997; Nyström *et al*, 1999; Bodek, 2000; Hwang and Shin, 2000; Desbrieres, 2002; Anchisi *et al*, 2004; Martínez-Ruvalcaba *et al*, 2004; Fernandez *et al*, 2006; Mironov *et al*, 2007). For instance, Kienzle-Sterze *et al*. (1985) demonstrated that the viscosity of concentrated chitosan solutions increases with increasing chitosan concentration and that a shear thinning behavior is observed at polymer concentrations above 0.50 g dL^{-1}. They also reported that the zero shear viscosity is independent of the ionic strength while it is dependent on pH of the media.

Wang and Xu (1994) reported that the non-Newtonian behavior of chitosan solutions increases with increasing the degree of deacetylation (DD) attributed to the chains expanded structure and the increase of entanglements. Mucha (1997) also reported an increase in the shear stress and viscosity of chitosan solutions with increasing chitosan concentration due to the increase of the chain entanglements between the macromolecular chains.

The rheology of two aqueous systems of chitosan, unmodified chitosan (UM-chitosan) and hydrophobically-modified chitosan (HM-chitosan), was studied by Nyström *et al*. (1999). Results revealed that the linear and non-linear viscoelasticity are affected by several factors such as pH, temperature, amount of surfactant and polymer concentration. However, the HM-chitosan was found to be heavily dependent on these factors. In addition, the shear thinning behavior was found to be pronounced at higher shear rates for both systems.

Hwang and Shin (2000) revealed that the shear rate dependence of viscosity of chitosan solutions was more remarkable at higher chitosan concentrations.

Bodek (2000) studied the rheological properties of microcrystalline chitosan hydrogels prepared by adding a methylcellulose hydrogel to aqueous chitosan dispersions. It was found that depending on the kind and content of the pharmaceutical substances as well as the interactions between the polymer and the pharmaceutical substances, a pseudoplastic system can be observed.

Martínez-Ruvalcaba *et al*. (2004) studied the influence of temperature, acid type and addition of salt on the steady-shear rheology of concentrated chitosan solutions. Viscosity and normal stress were decreased with increasing temperature and decreasing chitosan concentration. In addition, chitosan dissolved in hydrochloric acid solutions exhibited lower steady-shear viscosity and normal stress than in other acids solutions. However, salt addition was found to be the most effective parameter on the rheology of chitosan solutions.

Anchisi *et al*. (2004) found that rheological properties of chitosan dispersions are affected by the molecular weight and that all chitosan dispersions showed a pseudoplastic and shear thinning behavior. It was also found that the presence of glycols leads to decreasing in the apparent viscosity compared to the corresponding base dispersions.

The formation of chitosan hydrogels in the acetic acid-water-propanediol medium was reported by Montembaulta *et al*. (2005). The gelation at different conditions of the polymer concentration, the degree of acetylation (DA) of chitosan and the composition of the initial

solvent was studied by rheometry. It was found that the optimal gelation condition for cartilage regeneration application is DA = 40%, a water/alcohol ratio of 50/50 and a polymer concentration of 1.5%.

The production of pH-induced monolithic hydrogels through uniform neutralization of slightly acidic chitosan solutions with ammonia generated from enzymatic hydrolysis of urea was analyzed via rheology and reported by Chenite *et al*. (2006). A decrease in gelation time with an increase in temperature from 15 to 45^{o}C was found to be pronounced as a result of the synergistic influence of increased reactant diffusion and urease activity. It was also observed that the gelation is accelerated as the urea concentration increases up to a certain limit, and then a slow decrease in the gelation kinetics was noticed. This study indicated the possibility of using the autogelling solutions of chitosan as injectable gels for tissue engineering and drug delivery.

Fernandez *et al*. (2006) investigated the rheology of chitosan gels with different molecular weights (MW) i.e., low MW and medium MW. Non-Newtonian flow independent of the time was observed for the two gels, with a pseudoplastic behavior for the low MW chitosan and plastic behavior for the medium MW one. Mironov *et al*. (2007) reported a decrease in the dynamic viscosity of chitosan solutions in acetic acid during storage due to polymer degradation.

Rheology of crosslinked chitosan

Argüelles-Monal *et al*. (1998) reported an apparent yield stress at very low frequencies for chitosan dissolved in acetic acid solution. As the chemical cross-linkages were taking place, the weak self-associated network of chitosan was gradually replaced by a permanent

covalent network as the molar ratio of aldehyde/amine groups, *R*, is increased. They also reported that a strong permanent gel was formed at higher cross-linking levels.

Moura *et al*. (2007) reported the rheology of chitosan solutions crosslinked with a natural crosslinker, genipin at various concentrations. Results showed that stronger elastic gels of the crosslinked solutions were obtained under physiological conditions compared to those of the pure component. They also reported that the values of the gelation time obtained from the crossover of G'' (loss modulus) and G' (storage modulus) and that recorded by the Winter–Chambon criterion were in excellent agreement.

Rheology of chitosan mixture solutions and gels

The rheological characteristics of mixture solutions of chitosan and polyoxyethylene (POE) with various MWs were reported (Nikolova *et al*, 1998). Pseudoplastic non-Newtonian behavior was observed for each of the pure polymer solutions as well as for their mixture solutions. It was assumed that the rheological behavior of the CS/POE mixtures is dictated by the content of CS in the mixture.

The rheological properties of a semi-interpenetrating chitosan–PEO network were reported by Khalid *et al*. (1999). Results showed the elastic properties were increased by the semi-interpenetration due to the presence of the polyethylene oxide physical network.

Jiang *et al*. (1999) investigated the phase transition behavior with variation in temperature and frequency of water within the chitosan hydrogels using oscillatory shear rheology. The results showed that the water phase transition, which occurred with a decrease in temperature, had a significant influence on all the measured viscoelastic properties (shear

storage modulus, shear loss modulus and shear loss tangent). The changes of the viscoelastic properties were found to be related to the mobility of water within the gels.

The preparation of neutralizing highly deacetylated chitosan solutions (at physicological pH) with β-glycerol phosphate was reported by Chenite *et al*. (2001). Rheological measurements demonstrated that the hydrogel formation with a subsequent heating of these solutions was quickly obtained. The results also showed a pH-sensitivity and a temperature–dependence for the sol/gel transition temperature and gelling time respectively. Based upon the observations obtained, multiple interactions between chitosan, glycerol phosphate and water were suggested for the molecular mechanism of the gelation.

Salomé Machado *et al*. (2002) reported the preparation and characterization of collagen: chitosan blends in 1:1 ratio by rheological studies. Results showed that a decrease of storage modulus, viscous loss modulus and apparent viscosity (as a function of frequency) was observed with the addition of chitosan. It was also found that collagen/chitosan blends present a more fluid-like viscoelastic behavior than solid-like one.

The rheological properties of kaolin/chitosan aqueous dispersions were characterized by Bezerril *et al*. (2006). The kaolin/chitosan dispersions showed a pseudoplastic behavior, which was increased at lower shear rate. The authors related the increase in pseudoplasticity to a higher occurrence of particle-polymer-particle interactions due to the adsorption of chitosan macromolecules on the surface of kaolin particles. However, the rheological behavior of these dispersions could not be described by a simple power law.

The rheological properties of chitosan and xanthan hydrogel have been studied by Martìnez-Ruvalcaba *et al*. (2007). Results show that chitosan/xanthan hydrogels behave like weak gels. Almost linear increase in the shear modulus was observed with a frequency in the range between 0.1 and 65 s^{-1}. It was also found that factors, such as hydrogel concentration and nature of dispersion, play a significant role in the final structure and properties of the hydrogels. The viscoelastic properties of chitosan/PVA blend hydrogel were investigated rheologically by Tang *et al*. (2007). Results indicated good mechanical strength of the gel.

The rheological properties of a mixture of lecithin/chitosan vesicles by means of shear stress against shear rate measurements were investigated by Madrigal-Carballo *et al*. (2008). The results showed that chitosan can promote the transition of planar sheets into closed structures, such as vesicles. It was also found that this system suggests a thixotropic behavior.

Chitosan and glycerol-2-phosphate (b-GP) mixtures were prepared and analyzed by Kempe *et al*. (2008). The rheological properties were studied using the oscillating rheology for characterizing the macroviscosities of the sol and gel systems. It was found that an amount of 6% b-GP was necessary to induce the gel formation and that neither the gelation process nor the chitosan/b-GP proportions had an effect on the pH to a significant amount.

Wanchoo *et al*. (2008) investigated the miscibility of chitosan blends with hydrophilic polymers; chitosan/PVA chitosan/poly vinylpyrollidone (CS/PVP) and chitosan/poly (ethylene oxide) (CS/PEO). Viscosity and Rheological measurements showed that

rheograms of all the blends were found to lie between the rheograms of pure components over the entire compositional range indicating that the miscibility of the blends may occur.

The rheological study of chitosan/gelatin composite has been already investigated by Wang *et al*. (2009). Results showed that the formation of complex between chitosan and gelatin was mainly through hydrogen bond and that a close relation between the interactions between the two polymers in solution and the mechanical properties of the formed films is found. The authors concluded the possibility of designing films with desired mechanical properties through the combination of different polymers at optimum weight ratios.

The preparation of chitosan ferro-gels was reported by Herandez *et al*. (2009). The method used was the simultaneous co-precipitation of iron ions in alkali media and chitosan gelation. As far as the viscoelastic modulus measurements are concerned, the reinforcement of the chitosan ferrogels was achieved in the presence of magnetite nanoparticles, which was observed by the increase of the viscoelastic modulus.

2.3 Blends of chitosan

2.3.1 Introduction

Polymer blending is a method that is commonly used for providing desirable polymeric materials with combined properties for particular applications (Taravel and Domard, 1996). Recently, blends of natural polymers have been becoming considerably important due to their strong potential in replacing synthetic polymers in many applications (Wood, 2001) in addition of being renewable resources, nontoxic, inexpensive and leave biodegradable waste (Neto *et al*, 2005). Among natural polymers, chitosan and its blends occupies a special position due its versatility and suitability of large number of applications as

discussed earlier. Investigation of blends of chitosan with synthetic and naturally occurring macromolecules has attracted much attention in the recently years in various occasions (Mucha, 1998; Srinivasa *et al*, 2003; Shanmugasundaram *et al*, 2001; Chen *et al*, 2002; Sionkowska *et al*, 2004).

Like other polymer blends, the properties of chitosan blends depends upon the miscibility of its components at the molecular scale, which is attributed to specific interactions between polymeric components. The most common interactions in the chitosan blends are: hydrogen bonding, ionic and dipole, π-electrons and charge-transfer complexes. Various techniques such as thermal analysis (Cabanelas *et al*, 2005), electron microscopy (Olabisi *et al,* 1979*)*, viscometric measurements (Jiang and Han, 1998) and dynamic mechanical studies (Lewandowska, 2005), have been used to investigate the polymer–polymer miscibility in solution or in solid state.

The purposes of chitosan blending vary depending upon the application demands. This includes : 1) to enhance hydrophilicity (Kweon and Kang, 1999; Zhang *et al*, 2002) 2) to enhance mechanical properties (Arvanitoyannis *et al*, 1998; Isogai and Atalla, 1992; Lee *et al*, 1999; Park *et al*, 1999; Ratto *et al*, 1996), 3) to improve blood compatibility (Amiji, 1995; Chandy and Sharma, 1992; Ishihara *et al*, 2002) and to enhance antibacterial properties (Wu *et al*, 2004). In blood contact applications, chitosan promotes surface-induced thrombosis and embolization (Amiji, 1995; Chandy and Sharma, 1992; Ishihara *et al*, 2002).

The selection of the polymers to be blended with the chitosan depends on the property to be conferred or boosted. For example the hydrophilic property of chitosan is modified by

blending with polymers such as PEG and PVA (Kweon and Kang, 1999; Zhang *et al*, 2002). Chitosan was also blended with several polymers such as polyamides, poly (acrylic acid), gelatin, silk fibroin and cellulose to enhance mechanical properties (Arvanitoyannis *et al*, 1998; Isogai and Atalla, 1992; Lee *et al*, 1999; Park *et al*, 1999; Ratto *et al*, 1996). To enhance antibacterial properties, chitosan is blended with cellulose (Wu *et al*, 2004).

2.3.2. Preparation of chitosan blended solutions and films

Generally, there are two main methods that are commonly used in blending of chitosan: 1) dissolving in a solvent followed by evaporation (solution blending) (Singh and Roy, 1997; Yoshikawa *et al*, 1998) and 2) mixing under fusion conditions (melt blending) (Correlo *et al*, 2005). However, according to the literature, solution blending is the most applied method for preparing chitosan blends due to its simplicity and suitability for producing various forms of blends (beads, microspheres, films and fibers). Figure 2.5 shows a schematic representation of types of chitosan blends and methods of blending.

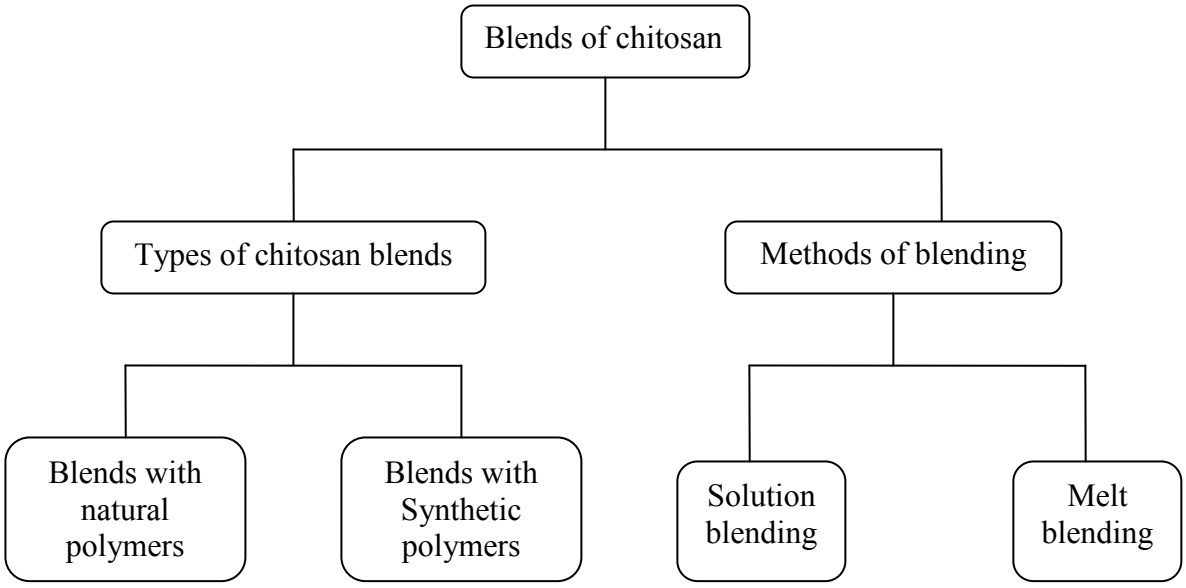


Figure 2.5: Schematic representation of methods of preparation of chitosan blends and their types.

In solution blending, chitosan is dissolved in an appropriate solvent (usually diluted acetic acid) with continuous stirring at room temperature. This is followed by mixing a desired amount of another polymer after being dissolved in a solvent under continuous stirring conditions. The blend solution of chitosan is often crosslinked by addition of acrosslinking agent to improve mechanical properties. Subsequently, the blend solution is filtered and then cast on a glass plate or a petri dish and left to dry under room or oven temperature. Eventually, the blend is washed with NaOH solution to remove the excess acetic acid. Table 2.1 presents a summary of the preparation of some chitosan blends using the casting method.

Table 2.1: Summary of the preparation of chitosan blend films by the casting technique suggested by some authors.

Chitosan source /(DD)	Solvent used	Blend	Crosslinker	Plasticizer	Film thickness	Ref.
Shrimp/85%	1%(v/v) acetic acid	CS/glycerol CS/sorbitol CS/PEG CS/tween60 CS/tween 80	_	_	2.82-3.95×10^{-2}	(Miranda *et al*, 2004)
91%	1%(w/v) acetic acid	CS/gelatin	_	_	_	(Yuan and Wei, 2004)
_	2.0 wt% acetic acid	CS/cellulose	_	_	_	(Wu *et al*, 2004)
Crab/80-85%	1% acetic acid	CS/PLA	_	_	_	(Suyatma *et al*, 2004)
Shrimp/80%	2% acetic acid	PA/CS	Glutaraldehyde	_	_	(Thanpitcha *et al*, 2006)
Crab/> 85%	1% acetic acid	CS/gelatin	_	_	_	(Cheng *et al*, 2003)
56%	5% acetic acid+1% HCl (catalyzer)	HPC/CS	Glyoxal and glutaraldehyde	_	20-30μm	(Suto and Ui, 1996)
96% (PG)	Lactic acid	CS/corn starch CS/dextran	glutaraldehyde	Propylene glycol (PG)	_	(Wittaya-areekul and Prahsarn, 2006)
60%	(0.2 M sodium acetate +0.1 N acetic acid)	CS/gelatin	_	_	_	(Basavaraju *et al*, 2006)
95.7%	1% acetic acid	CS/CE	_	_	_	(Yin *et al*, 2006)
90%	1% (v/v) lactic acid	CS/starch	_	Glycerin	~ 2.54 μm	(Xu *et al*, 2005)
_	2% acetic acid	CS/PVA	_	Sorbitol and sucrose		(Arvanitoyannis *et al*, 1997)
91%	2% acetic acid	CS/PVP CS/PEG	_	_	40-60 μm	(Zeng *et al*, 2004)
85%	0.5M acetic acid	CS/ collagen	_	_	_	(Ye *et al*, 2007)
~ 84%	trifluoroacetic acid (TFA)	CS/nylon 11	_	_	_	(Kuo *et al*, 2006)
85, 87 and 93%	4 wt% acetic acid	(P*N*V2P)/CS	_	_	40-50 μm	(Caykara *et al*, 2006)
84%	Formic acid	CS/nylon 66	sulfuric acid	_	_	(Smitha et al, 2006)

~ 85%	o.5M acetic acid	CS/ PCA	_	_	50–60 µm when dry and 90–120 µm	(Sarasam and Madihally, 2005)
_	88 wt% formic acid	chitosan/ NMN6	1M H2SO4	_	20–40µm	(Shieh and Huang, 1998)

There are several factors that can influence the properties of chitosan blend films among which are the chitosan source and the film thickness which are usually neglected in the literature as can be seen in Table 2.1.

2. 3.3 Blends of chitosan with synthetic polymers

Blending of chitosan with synthetic polymers is a convenient method for preparation of synthetic biodegradable polymers having versatile properties such as good water absorbance and enhanced mechanical properties (e.g. synthetic biodegradable polymers used as biomaterials range in tensile strength from 16 to 50 MPa and modulus from 400 to 3000 MPa) while maintaining biodegradability (Engleberg and Kohn, 1991). Because of the large applications of chitosan in various fields, blends with synthetic polymers having wide range of physicochemical properties have been prepared in various occasions with solution blending investigated by many workers.

Polyvinyl alcohol and polyethylene are among synthetic polymers that have been frequently blended with chitosan (Blair *et al*, 1987; Suto and Ui, 1996; Mucha, 1998; Srinivasa *et al*, 2003). These blends represent new materials possessing better hydrophilicity, mechanical properties and biocompatibility than the characteristics of single components (Cascone, 1997; Xiao *et al*, 2000). The physico-chemical properties of chitosan/poly (vinyl alcohol) (PVA) blends using sorbitol and sucrose as plasticizers were investigated by Arvanitoyannis *et al.* (1997). Melting point and heat of fusion showed a

decreasing trend with increasing the plasticizer content. However, the percentage of elongation together with CO_2 and water vapor permeability of the blends showed an increase with increasing the plasticizer content coupled with a proportional decrease in the tensile strength and the modulus were observed.

Graft copolymerization of chitosan with acrylonitrile, methylmethacrylate (MMA), methacrylic acid, 2-hydroxyethylmethacrylate (HEMA), and acryl-amide has been reported in the literature. Similarly, styrene, vinyl acetate, acrylamide, MMA, and HEMA, have also been grafted on chitosan. Grafting of chitosan with *N*,*N*′-dimethylaminoethylmethacrylate (DMAEMA) has also been reported (Singh and Roy, 1997; Singh and Roy, 1994; Blair *et al*, 1987; Lagos and Reyes, 1988; Shigeno *et al*, 1982). Blends of chitosan with polyurethane were prepared using solvent casting method and their mechanical properties were evaluated (Gong *et al*, 1998). Poor phase properties were reported.

Hybrid materials of chitosan with polynosic (viscose rayon) were generated by a mechanical blending method. Chitin and chitosan were reacted with 1,6-diisocyanatohexane (poly-urea (urethanes)) in DMA-LiCl solutions and their properties evaluated (Muzzarelli *et al*, 1994).

Biodegradable films of chitosan blend containing polyethylene glycol (PEG) or polyvinyl alcohol (PVA) were prepared by mixing PEG or PVA with a solution of chitosan acetate, and films were prepared by the casting method (Engleberg and Kohn, 1991; Chandy and Sharma, 1992). Homogenous films with increased value of initial temperature of thermal degradation were produced.

Chitosan blended films containing glycerol (0.25% and 0.5%) were prepared by Butler *et al*. (1996). Oxygen and ethylene permeability remained constant during the storage period, but percent elongation decreased.

Shieh and Huang (1998) have reported the preparation and characterization of chitosan/ N-methylol nylon 6 blends in form of membranes for the separation of ethanol-water mixtures by pervaporation. The obtained blend membranes were treated with H_2SO_4 to enhance their separation performance. The blend composition was found to play a significant role in affecting the performance of the membranes.

Wiles *et al*. (2000) investigated the mechanical properties of chitosan and polyethylene glycol (PEG) films. It was revealed that the obtained blend films containing PEG of 0.25% and 0.5% resulted in an increased percent of elongation, but tensile strength (TS) and water vapor transmission rate (WVTR) were decreased.

The mechanical properties of chitosan films prepared by blending with polyols (glycerol, sorbitol and polyethylene glycol and fatty acids, stearic and palmitic acids) were studied by Srinivasa *et al.* (2003). Investigations of the mechanical properties showed a decrease in the tensile strength with the addition of polyols and fatty acids, while the percent of elongation was increased in polyol blend films. However, fatty blend films didn't show any significant difference.

Attempts to prepare film blends of chitosan with poly (lactic acid) (PLA) were reported by Nugraha *et al*. (2004). The thermal and mechanical properties of the obtained films

revealed that chitosan/PLA blends are incompatible, i.e. there was no interaction between the two polymers.

Chitosan blends with polyvinyl pyrrolidone (PVP) and polyethylene glycol (PEG) were reported by Zeng *et al*. (2004). The compatibility of the blends was proved by FTIR, wide angle x-ray diffraction (WAXD) and differential scanning calorimeter (DSC) analysis. The chitosan/PVP blend showed no porosity unlike chitosan/PEG blend, which showed high porosity. The former and latter are attributed to the strong and weak interactions in the blends, respectively.

Sandoval *et al*. (2005) studied the compatibility of two chitosan blends with (vinyl alcohol) (PVA) and poly (2-hydroxyethyl methacrylate) (P2HEM) through molecular dynamic simulations. The aim of this study was to find out which of the two functional groups, (–CH_2OH) and (–CH_2OH), of chitosan is responsible of the interaction. It was concluded that the interaction occurs predominantly with the hydroxymethyl groups of chitosan at low composition of P2HEM and PVA while the interaction with the amine groups increases with the increase in the composition of the two polymers.

Sarasam and Madihally (2005) have reported the preparation and characterization of chitosan/polycaproactone (PCA) blends at different ratios, temperatures, and humidities, for tissue engineering applications. According to the thermograms (using Flory–Huggins theory), the two polymers were successfully blended. Measurements of differential scanning calorimetry (DSC) also indicated that there is an interaction between the two components when the results were analyzed using Nishi–Wang equation. However, no remarkable alterations relative to chitosan were obtained by tensile properties

measurements while significant improvements were observed after solvent annealing. In addition, significant improvement in mechanical properties was achieved when a 50:50 ratio blend dried at 55^{o}C was used. It was concluded that such a blend has a potential for various applications in tissue engineering.

Chitosan was melt blended with poly-ε-caprolactone (PCL), poly (butylene succinate) (PBS), poly (lactic acid) (PLA), poly (butylene terephthalate adipate) (PBTA), and poly (butylene succinate adipate) (PBSA) (Correlo *et al*, 2005). For the chitosan/PBS blend, the amount of chitosan was varied from 25% to 70% by weight. The remaining polyesters had 50% of chitosan by weight. Addition of chitosan to PBS or PBSA tends to depress the melting temperature of the polyester. The crystallinity of the polyesters (PCL, PBS, PBSA) containing 50% chitosan decreased. Adding chitosan to the blends decreased the tensile strength but increased the tensile modulus. Chitosan displayed intermediate adhesion to the polyester matrix. Microscopic results indicated that the skin layer is polyester rich, while the core is a blend of chitosan and polyester. Fractured surface of chitosan blended with a high T_g polymer, such as PLA, displayed a brittle fracture. Blends of chitosan with PCL, PBTA, or PBSA display fibrous appearances at the fractured surface due to the stretching of the polymer threads. Increasing the amount of chitosan in the blends also reduced the ductility of the fractured surface. The chitosan phase agglomerated into spherical domains was clustered into sheaths. Pull-out of chitosan particles is evident in tensile-fractured surfaces for blends of chitosan with ductile polymers but absent in the blends with PLA. PBS displays a less lamellar orientation when compared to PCL or PBSA. The orientation of the polyesters (PCL, PBSA) does not seem to be affected by the addition of chitosan

Chitosan/nylon 11 blends at different ratios were prepared and characterized by FTIR, scanning electron microscopy (SEM) and x-ray (Kuo *et al*, 2006). Biodegradability was also investigated. Results revealed that physical properties of nylon 11 were greatly affected by the addition of chitosan in the blended films and that good biodegradability of the resulting blends was observed.

Smitha *et al*. (2006) have reported the preparation and characterization of the crosslinked blends of chitosan/nylon 66 at different weight compositions. The crosslinking and thermal stability of the blends after crosslinking were confirmed by FTIR and TGA, respectively. The obtained results showed good indication for dehydration of dioxane and moderate water sorption (50–90%) of the blends with no significant effects on mechanical stability of the blends. Increasing water concentration in feed brought about an improved membrane swelling and thereby improved flux at reduced selectivity. Varying thickness resulted in a remarkable lowering of the flux with some improvement in selectivity. Higher permeate pressures caused a reduction in both the flux and selectivity.

Various proportions of three chitosan portions having different molecular weights were blended with poly (N-vinyl-2-pyrrolidone) (P*N*V2P) (Caykara *et al*, 2006). The surface properties of the films were studied by scanning electron microscopy (SEM) and contact-angle measurements. It was revealed that the blend surfaces were enriched in low surface free energy component, *i.e.*, chitosan.

Other studies on chitosan blends and composites were reported in the literature. For example, chitosan/polyethylene glycol (Mucha *et al*, 1999), chitosan/polyethylene oxide

(Khoo *et al*, 2003), chitosan/polyvinylpyrrolidone (Abou-Aiad *et al*, 2006), chitosan/PVA/gelatin (Chen *et al*, 2008b) and chitosan/PVA/pectin (Tripathi *et al*, 2010).

2.3.4 Blending of chitosan with natural polymers

Blending of chitosan with other natural polymers has been proposed as an interesting method to bring about new bio-materials of improved properties to meet the requirements of specific applications. Reports on blending of chitosan with natural polymer have been published in various occasion. For example, a number of publications have been reported blending of chitosan with collagen (Thacharodi and Rao, 1995; Thacharodi and Rao, 1996; Hirano *et al*, 2000; Shanmugasundaram *et al*, 2001; Chen *et al*, 2002; Sionkowska *et al*, 2004). The effect of chitosan on the properties of collagen has also been investigated (Taravel and Domard, 1993; Taravel and Domard, 1996; Salome Machado *et al*, 2002). These studies have shown that chitosan has the ability to modify the mechanical properties of collagen.

Arvanitoyannis *et al*. (1998) developed films of chitosan and gelatin by casting from their combined aqueous solutions (pH 4.0) at 60°C and evaporating at 22 or 60°C (low- and high-temperature methods, respectively). The thermal, mechanical and gas/water permeation properties of these composite films, plasticized with water or polyols, were investigated. An increase in the total plasticizer content resulted in a considerable decrease of elasticity modulus and tensile strength, whereas the percentage of elongation increased. It was also found that the low-temperature preparation method led to a higher percentage of renaturation (crystallinity) of gelatin, therefore a decrease, by 1-2 orders of magnitude, of CO_2 and O_2 permeability in the chitosan/gelatin blends was observed. An increase in the

total plasticizer content (water or polyols) of these blends was found to be proportional to an increase in their gas permeability.

Ikejima *et al*. (1999) reported the development of blended films of microbial poly (3-hydroxybutyric acid) (PHB) with chitin and chitosan as completely biodegradable polyester/polysaccharide composites. DSC measurements revealed that crystallization of PHB in these blends was suppressed when the proportion of polysaccharide increased. The same tendency was evident from the FTIR band intensity of the carbonyl stretching absorption originated from PHB. Chitosan was found to have a stronger ability to suppress the crystallization of PHB than with chitin. PHB in the blends was found (by ^{13}C NMR spectroscopy) to be trapped in the 'glassy' environment of the polysaccharide. Upon blending with PHB, the chitosan resonances were significantly broadened as compared to the chitin resonances. It was suggested that hydrogen bonds may be formed between the carbonyl groups of PHB and the amide -NH groups of chitin and chitosan. The crystallization behavior and environmental biodegradability were also investigated for the films of poly (3-hydroxybutyric acid) (PHB) blends with chitin and chitosan (Ikejima and Inoue, 2000). The blend films showed XRD peaks that arose from PHB crystalline component. It was suggested that the lamellar thickness of the PHB crystalline component in the blends was large enough to show detectable XRD peaks, but was too small to show an observable melting endotherm in the DSC thermogram and a crystalline band absorption in the FTIR spectrum. In the PHB/chitin and PHB/chitosan blends, thermal transition temperatures of PHB amorphous regions observed by dynamic mechanical thermal analysis were almost the same as that of neat PHB for both PHB/chitin and PHB/chitosan blends. It was also observed that both blended films tend to biodegrade in an environmental medium.

Preparation of chitosan/κ-carrageenan blends in form of films has been studied. The effect of type of solvent system (acetic, lactic, citric, maltic and ascorbic acids) on the mechanical properties on the obtained films was investigated (Park *et al*, 2001b). Ascorbic acid was found to increase the tensile strength and the percent of elongation of the films compared to other diluting acids. Results showed that there are interactions among the organic acids and κ-carrageenan and chitosan.

Other kinds of aqueous blends based on chitosan were also reported. Kweon *et al*. (2001) reported the preparation of aqueous blends of chitosan with protein. *Antheraea pernyi* silk fibroin (SF)/chitosan blended films were prepared by mixing aqueous solution of *A*. *pernyi* SF and acetic acid solution of chitosan. The conformation of *A*. *pernyi* SF in blended films was revealed to be a *β*-sheet structure, mainly due to the effect of using acetic acid as a mixing solvent. Blending with *A*. *pernyi* SF can enhance the thermal decomposition stability of chitosan.

The phase structure of poly (*R*)-(3-hydroxybutyrate) (PHB)/chitosan and poly (*R*)-(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (P(HB-*co*-HV))/chitosan blends using ^{1}H CRAMPS (combined rotation and multiple pulse spectroscopy) was investigated by Cheung *et al*. (2002). A modified BR24 sequence that yielded an intensity decay to zero mode rather than the traditional inversion-recovery mode was used to measure ^{1}H T_1. Single exponential T_1 decay is observed for the *β*-hydrogen of PHB or P (HB-*co*-HV) at 5.4 ppm and for the chitosan at 3.7 ppm. T_1 values of the blends are either faster than or intermediate to those of the plain polymers. The $T_{1\rho}$ decay of *β*-hydrogen is bi-exponential. The slow $T_{1\rho}$ decay component is interpreted in terms of the crystalline phase of PHB or P (HB-*co*-HV). The degree of crystallinity decreases with increasing wt% of chitosan in the

blend. The fast $T_{1\rho}$ of β-hydrogen and the $T_{1\rho}$ of chitosan in the blends either follow the same trend as or are faster than the weight-averaged values based on the $T_{1\rho}$ of the plain polymers. Depending on the observation obtained by DSC of a melting point depression and one effective T_g in the blends, it was strongly suggested that chitosan is miscible with either PHB or P(HB-*co*-HV) at all compositions.

Lazaridou and Biliaderis (2002) studied the thermo-mechanical properties of aqueous solution/cast films of chitosan, starch/chitosan and pullulan/chitosan using dynamic mechanical thermal analysis (DMTA) and large deformation tensile testing. Incorporation of sorbitol (10% and 30% d.b.) and/or adsorption of moisture by the films resulted in substantial depression of the glass transition (T_g) of the polysaccharide matrix due to plasticization. For the composite films there was no clear evidence of separate phase transitions of the individual polymeric constituents or a separate polyol phase; a rather broad but single drop of elastic modulus, E', and a single tan δ peak were observed. Tensile testing of films adjusted at various levels of moisture indicated large drops in Young's modulus and tensile strength (σ_{max}) with increasing levels of polyol and moisture; the sensitivity of the films to plasticization was in the order of starch/chitosan>pullulan/chitosan>chitosan. Modeling of the modulus data with Fermi's equation allowed comparison among samples for the fall in modulus around the glass transition zone as a function of moisture content under isothermal conditions.

A series of chitosan/gelatin blended films has been reported in various occasions (Cheng *et al*, 2003). Good compatibility and an increase in the water uptake of chitosan films were observed. The chitosan/gelatin blend showed higher percentage of elongation–at-break together with a lower Young's modulus. In addition, the average water contact angles of the

obtained films were found to be 60^{o}. The investigation of chitosan/gelatin films for oxygen permeability, optical transmittance, water absorptivity and mechanical properties was also reported by Yuan and Wei (2004). The obtained results showed an increase in water absorption and improvement in oxygen and solute permeability of the composite film of chitosan. The chitosan/gelatin films were found to be more transparent, flexible and biocompatible.

Zhai *et al*. (2004) reported the preparation of antibacterial chitosan/starch blended films. The chitosan/starch-blended films were prepared by irradiation of compression-molded starch-based mixtures in physical gel state with an electron beam at room temperature. After the incorporation of 20% chitosan into the starch film, the tensile strength and the flexibility of the starch film were significantly improved. In addition, an interaction and microphase separation between the starch and chitosan molecules was observed by the X-ray diffraction (XRD) and SEM analyses of the blended films. Furthermore, the starch/chitosan blended films were irradiated to produce a kind of antibacterial films. After irradiation, there was almost no change in the structure of the starch/chitosan blended films. However, antibacterial activity was induced even when the content of chitosan was only 5%, due to the degradation of chitosan in blend films under the action of irradiation.

Wu *et al*. (2004) studied the preparation and characterization of membranes of chitosan and cellulose blends using trifluoroacetic acid as a solvent. As far as the mechanical and dynamic mechanical thermal analyses are concerned, the cellulose/chitosan blends are almost incompatible. In addition, results obtained from water vapor transpiration rate and antibacterial measurements suggested the possibility of using the membranes of chitosan and cellulose as a wound dressing with antibacterial properties.

Suyatma *et al*. (2004) reported preparation of biodegradable blended films of chitosan with polylactic acid by solution mixing and film casting. The incorporation of polylactic acid with chitosan improved the water barrier properties and decreased the water sensitivity of the chitosan films. However, the tensile strength and elastic modulus of chitosan decreased with the addition of polylactic acid. Measurements of mechanical and thermal properties revealed that chitosan and polylactic acid blends are incompatible. This was found to be consistent with the results of FTIR analysis that showed the absence of specific interaction between chitosan and polylactic acid.

Mucha and Pawiak. (2005) have reported that films made from chitosan blends with hydroxylpropyl cellulose (HPC) resulted in good miscibility of components with better optical transparency and mechanical properties. The phase separation of the components occured more drastically after water removal due to the active compatibilization behavior of the water molecules in this system, which lead to the formation of additional hydrogen bonds.

Chitosan and starch blended films were prepared and characterized by Xu *et al.* (2005). The obtained films showed a decrease in the water vapor transmission rates and an increase in the tensile strength and the elongation-at-break with increasing starch ratio in the blend. FTIR spectroscopy and x-ray diffraction have suggested the existence of interaction and compatibility of the two film-forming components.

Wittaya-areekul and Prahsarn. (2006) have investigated the possibility of incorporation of corn starch and dextran with chitosan using glutaraldehyde a crosslinker for use in wound

dressing application. The effect of adding polypropylene glycol (as a plasticizer) on the chitosan blends was also reported. Results showed that corn starch and dextran can be incorporated into chitosan film to improve the physical strength, *i.e*., vapor penetration, water uptake, and oxygen penetration properties. The addition of propylene glycol was found to improve the film elasticity and all other properties mentioned already with exception of bio-adhesive properties.

Yin *et al*. (2006) have examined the miscibility of chitosan blends with two cellulose ethers, hydroxypropylmethylcellulose and methylcellulose by infrared spectroscopy, thermal gravimetric analysis, wide-angle X-ray diffraction and scanning electron microscopy. It was concluded that full miscibility cannot be achieved if the hydrogen bonding between the polymers is weak.

The miscibility of chitosan/gelatin blend in a buffer solution (0.2 M sodium acetate and 0.1 N acetic acid) has been assessed by means of viscosity, ultrasonic and refractive index method at 30 and 50^{o}C (Basavaraju *et al*, 2006). The obtained data indicated that the blend is immiscible in all proportions and that the variation of temperature has no influence on miscibility of the blend.

The compatibility of chitosan/collagen blends has been evaluated by dilute solution viscometry (Ye *et al*, 2007). The obtained data showed that collagen/chitosan blends are miscible at any ratio in acetic acid solutions at 25^{o}C. As far as the "memory effect'' is concerned, the blends are also miscible in the solid state.

2.3.5 Physical forms of chitosan blends

Chitosan blends exist in various physical forms including resins, microsphers, hydrogels, membranes and fibers. The selection of one particular physical form depends mainly on the system configuration to be used for particular applications. The process of shaping chitosan blends into desired physical form starts from mixing the blend components in the liquid form and applying the appropriate shaping method.

Resins (beads)/microspheres

Chitosan beads have been prepared by various methods, including solvent evaporation, coacervation and emulsion methods (Peniche *et al*, 2003; Genta *et al*, 1997). However, smaller and uniform beads can be obtained by the emulsion technique (Lim *et al*, 1997). Chitosan and its blends beads have been widely researched (Huguet *et al*, 1994, Polk *et al*, 1994; Liu *et al*, 1997; Dumitriu and Chornet, 1998, Murata *et al*, 1999; Tomoaki *et al*, 2000; Gonz´alez-Rodr´ıguez *et al*, 2002; Murata *et al*, 2002). The chitosan/alginate microparticles (Kim and Rha, 1989a,b), chitosan/xanthan microspheres (Chellat *et al*, 2000a) and chitosan/gelatin microspheres (Yao *et al*, 1996; Yuji *et al*, 1996) have also been reported.

A novel natural polymer chitosan/cellulose blend beads were prepared via homogeneous dissolution of chitosan and cellulose in methylmorpholine-*N*-oxide. The blend microspheres were prepared by spray-drying process have a spherical geometry and a smooth surface morphology (Twu *et al*, 2003).

Hydrogels

Hydrogels of biopolymer is a significant class of polymeric materials due to their interesting properties for potential application in various fields such as biomedical, pharmaceutical and environmental industries. Chitosan Hydrogels can be easily prepared from concentrated solution by re-precipitation under mild alkaline condition. In the recent years, interest has been increased on modification of chitosan and its blends by crosslinking to improve the hydrogel stability. Non-covalent cross-linked chitosan hydrgel blends have been reported in the literature, such as chitosan/alginate (Takka and Acarturk, 1999), chitosan/carboxymethyl-cellulose (Arguelles-Monal *et al*, 1993; Fukuda, 1980), chitosan/dextran sulfate (Sakiyama *et al*, 2001), chitosan/carboxymethyl-dextran (Fukuda and Kikuchi, 1978), chitosan/heparin (Kikuchi and Noda, 1976; Kratz *et al*, 1998), chitosan/carrageenan (Sakiyama *et al*, 1993), chitosan/pectin (Chang and Lin, 2000; Mireles *et al*, 1992), chitosan/collagen (Zhang *et al*, 1997), and chitosan/xanthan (Chelat *et al*, 2000b; Chu *et al*, 1995; Dumitriu and Chornet, 1996; 1998, 2000; Dumitriu *et al*, 1994; Ikeda *et al*, 1995). Crosslinking of chitosan hydrogel (Singh *et al,* 2006) and chitosan/PVA hydrogel membranes with formaldehyde (Yang *et al*, 2004) was also reported.

Films/membranes

It is well known that chitosan can be easily filmablized, especially, by a casting technique (Kanke *et al*, 1989; Bonvin and de Bertorell, 1993), which makes it useful for many applications including skin care, cosmetics, contact lenses, membranes and separators in various other technologies. Chitosan film properties have been reported by numerous authors (Butler *et al*, 1996; Chen and Hwa, 1996; Singh and Ray, 1998; Bégin and Van Calsteren, 1999). Chitosan films are clear, homogeneous and flexible with good oxygen barrier and mechanical properties (Hoagland and Parris, 1996; Kittur *et al*, 1998), but with

relatively low water vapor barrier characteristics (Butler *et al*, 1996). In addition, chitosan films are dense and do not possess pores (Muzzarelli, 1977; Hirano and Tokura, 1982). Since chitosan degrades before melting, it is necessary to dissolve it in an appropriate solvent prior to casting into films.

The properties of chitosan films depend on their morphology, which is affected by the molecular weight (Butler *et al*, 1996; Chen and Hwa, 1996), source of chitosan, degree of deacetylation, method of film preparation, free amine regenerating mechanism and most importantly by the type of dissolving solvent (Samuels, 1981; Blair *et al*, 1987; Lim and Wan, 1995). Acetic acid has been often used as a standard solvent for dissolving chitosan for film or membrane making. For example, Austin (1982) reported chitosan films making from 5% w/v chitosan solution dissolved in 4% v/v aqueous acetic acid followed by coagulatation in aqueous base solution. Averbach (1975) also reported casting chitosan films from 10% aqueous acetic acid solutions on stainless steel plates and drying them at 125^{o}C. Casting chitosan from other acid solutions such lactic acid yielded are softer and more pliable and bioadhesive films compared with those prepared from acetic acid (Khan *et al*, 2000).

Chitosan membranes prepared with various degrees of deacetylation of 75%, 87% and 96% (same molecular weight) were reported by Trung *et al*. (2006). Membranes obtained from chitosan of higher degree of deacetylation exhibited higher tensile strength and higher elongation at break. In addition, membranes casted from chitosan with 75% degree of deacetylation displayed higher permeability and higher water absorption.

Similar studies on chitosan membranes prepared from higher molecular weight chitosan showed has also higher tensile strength and percent elongation compared those obtained from low molecular weight ones (Chen and Hwa, 1996; Blair *et al*, 1987).

Preparation of blend or composite chitosan films associated with other bipolymers has been repeatedly reported in literature. Blend films such as chitosan/pectin laminated films (Hoagland and Parris, 1996), chitosan/cellulose (Hosokawa *et al*, 1990; Hasegawa *et al*, 1992b) and chitosan/methylcellulose films (Kittur *et al*, 1998) were investigated and improved properties compared to those of their pure components.

Similar films and membranes using blends of chitosan and synthetic polymers to improve the biocompatibility and expanded use as biomaterials were also reported in various occasions. For example, membranes prepared from blends of chitosan and polyvinyl alcohol (PVA) were investigated. The obtained membranes were found to be clear and homogeneous and have mechanical resistance is greater than that of the pure components (Miya *et al*, 1983; Miya and Iwamoto, 1984). Such membranes were capable of transport of halogenated ions and diffusion of cattle serum albumin (Uragami *et al*, 1983) and vitamin B_{12} (Nakatsuka *et al*, 1992) when crosslinked.

Fibers

Fiber is another physical form of chitosan, which was first reported as early as 1926 (Kunike, 1926). It possesses many advantages such as superior mechanical properties compared with the same material in bulk form. However, due to the high production cost, it was necessary for researchers to look into blends or composites of this polymer with other yarns. Fibers of chitosan blends have been frequently reported in literature. For instance,

fibers of chistosan blends with collagen (Chen *et al*, 2006, 2007; Chen *et al*, 2008a), starch (Wang *et al*, 2007), poly (ethylene oxide) (Bhattarai *et al*, 2005), poly (vinyl alcohol) (Jia *et al*, 2007; Zhou *et al*, 2006), silk fibroin (Park *et al*, 2004) and alginate (Liao *et al*, 2005) have been reported.

Sponge

Chitosan sponge is of high interest in drug carrier systems. This is due to chitosan biodegradability, biocompatibility, antibacterial activity and non-toxicity of chitosan together with film forming capability. However, chitosan solutions cannot be foamed alone. Therefore, it is often combined with another good foaming polymer such as gelatin, which is also a cheap biodegradable polymer with good foaming properties (Poole, 1989). When blended with chitosan, gelatin makes a good contact with chitosan and forms polyionic complexes of slower rate of dissolution than chitosan at the appropriate pH value (Thacharodi and Rao, 1995). Such property could be utilized in drug release to a wound while the spongy form absorbs the wound fluid (Oungbho and Müller, 1997). The release profile and biodegradability of both polymers can be effectively controlled by crosslinking (Tabata and Ikada, 1989; Jameela *et al*, 1998).

Similar sponges based on alginate and chitosan were also studied in various occasion (Shapiro and Cohen, 1997; Kofuji *et al*, 2001; Kataoka *et al*, 2001; Singla and Chawla, 2001; Kumar, 2000; Coppi *et al*, 2002; Lai *et al*, 2003). For example, chitosan and sodium alginate blended sponge incorporating silver sulfadizine was reported by Kim *et al*. (1999) for wound dressing application. Yang *et al*. (2001) also prepared hepatocyte-loaded alginate/galactosylated chitosan sponges for the growth of liver tissue.

2.3.6 Some applications of chitosan blends

According to the literature, most of the applications of chitosan blends are in the pharmaceutical and biomedical fields. For example, chitosan has been receiving increasing interest in drug delivery applications due to its capacity to enhance transport of hydrophilic drugs. It has also reported to be useful in colon- or nasal delivery. Chitosan is also of current interest as a carrier in gene delivery. Several chitosan nanocomposites were evaluated based on the ionotropic gelation, or numerous chitosan/DNA nanoparticles were framed from the complexation of the cationic polymer with DNA plasmid. Ionically interaction and modification only of chitosan as cationic polymer and anions result in these particles.

In the area of drug delivery systems, chitosan blends have been widely used for control release of drugs because of their advantageous properties such as non-toxicity, biocompatibility, biodegradability and availability of terminal functional groups (Chandy and Sharma, 1990). Various physical forms of chitosan blends such as microparticles (Berthold *et al*, 1996), tablets (Adusumilli and Bolton, 1991), films (Kanke *et al*, 1989), beads **(**Aral and Akbuga, 1998), gels (Kristl *et al*, 1993) were proposed various drug release applications and as an absorption enhancer for nasal and oral drug delivery (Illum *et al*, 1994). For example, various blends of collagen and chitosan were used for architecture of membranes for controlled release (Thacharodi and Rao, 1995; Thacharodi and Rao, 1996; Leffler and Müller, 2000). This also included proposing chitosan as a useful excipient for obtaining sustained release of water-soluble drugs and for enhancing the bioavailability of poorly water-soluble compounds. Moreover, chitosan has also been presented as a useful polymer for colon-specific and oral administered drug delivery (Tozaki *et al*, 1997). This has derived huge research efforts that led to publication of immense number of studies for a

wide variety of applications in the field of controlled release (over 2000 papers in the last 15 years), which makes it impossible to be reviewed here.

Tissues engineering field is another biomedical field where chitosan blends and other forms have found growing interest. Chitosan-based materials have been reported for tissue engineering in different physical forms, including porous scaffolds and gels (Madihally and Matthew, 1999; Suh and Matthew, 2000; Chung *et al*, 2002; Gutowska *et al*, 2001). For example, blends of collagen and chitosan have been used for the design of polymeric scaffolds for in vitro culture of human cells, tissue engineering skin and implant fibers (Shanmugasundaram *et al*, 2001; Ramay *et al*, 2005).

As an anti-microbial agent, chitosan is a biopolymer that has been well known as being able to accelerate the healing of wounds in human (Kojima *et al*, 1998). It was reported that chitosan stimulated the migration of polymorphonuclear (PMN) as well as mononuclear cells and accelerated the re-epithelialization and normal skin regeneration (Usami *et al*, 1994). The antimicrobial activity of chitosan is well known against a variety of bacteria and fungi coming from its polycationic nature (Roberts, 1992). The interaction between positively charged chitosan and negatively charged microbial cell wall leads to the leakage of intracellular constituents. The binding of chitosan with DNA and inhibition of mRNA synthesis occur via the penetration of chitosan into the nuclei of the microorganisms and interfering with the synthesis of mRNA and proteins. Furthermore, chitosan is a biomaterial widely used for effective delivery of many pharmaceuticals (Lorenzo-Lamosa *et al*, 1998). Accordingly, chitosan may be suitable for incorporating other antipyrotic for the preparation of long-acting antibacterial wound dressing.

In environmental applications, due to its powerful chelating ability, chitosan was found to be among the most powerful heavy metal ion binders (Krajewska, 2005). This is performed mainly with use of chitosan-based powders, flakes, gel beads, composite membrane or others (Kaminski and Modrzejewska, 1997; Taha *et al*, 1996; Genc *et al*, 2002). In general, the affinity of chitosan to heavy metal ions is as follows: Pd>Au>Hg>Pt>Cu>Ni>Zn>Mn>Pb>Co>Cr>Cd>Ag (Koshijima *et al*, 1973; Peter, 1995; Krajewska, 2001). Chitosan blends can be also applied for dehydration of solvents. For instance, it has been reported that chitosan/N-methylol nylon 6 blend membranes can be used for the separation of ethanol-water mixtures in terms of acid (H_2SO4) post-treatment, feed concentration, blend ratio and temperature (Shieh and Huang, 1998).

2.3.7 Contribution of the present study

Based on the previous studies on chitosan blends with natural and synthetic polymers, reports on chitosan binary blends with agar and ternary blends of chitosan with both of agar and PVA in the solid and liquid phases have not been revealed. Accordinly, the pesent work is to study three types of blends, i.e chitosan/agar, chitosan/PVA and citosan/agar/PVA with the aim to establish relationships between the proportions of the blend components on the rheological behavior in the liquid phase of the blends and the impact of that on the component miscibility. The properties of the obtained three chitosan based blends were investigated to elucidate the impact of the variation of the blends composition on their compatibility in the solid phase represented by blended films. A study of such kind can help in optimising the preparation conditions towards obtaining chitosan based blended films with improved properties to promote further applications. It also helps in estabishing and understanding composition-property correlations in the blended films.

3.0 Chapter 3: Experimental

3.1 Materials

Chitosan of a degree of deacetylation (DD) of 88.1% determined by UV method (Muzzarelli and Rochetti, 1985) was provided by the chitin-chitosan laboratory of Universiti Kebangsaan Malaysia (UKM) (Table 3.1). The agar was purchased from Sigma-Aldrich**.** PVA Fluka (56-98) with an average molecular weight of 195 x 10^3 g mol^{-1} was also used in this work. Acetic acid (glacial 100%, pro-analysi) was purchased from Merck (Darmstadt, Germany). Ultra pure water (Maxima Ultra Pure Water, Elga-Prima Corp, UK) with a resistivity greater than 18MΩ/cm was used to prepare all solutions. All chemicals were used without further purification and freshly prepared solutions were used in all experiments.

Table 3. 1: Specifications of chitosan used in this work.

Property	
Physical form	Flakes
Color	White
Ash content	0.30%
Water content	10-13%
Degree of deacetylation	88.1%
pH of water extract	7.63
Solubility in 1% acetic acid	Very soluble

3.2 Procedures

3.2.1 Preparation of the solutions

The chitosan was dried in an oven until a constant weight was observed. A 10 g L^{-1} solution of chitosan was prepared by dissolving 5 g of chitosan in 500 mL acetic acid (0.1M) followed by continous stirring and heating at 60^{o}C for 16 h. The solution was filtered to

remove dust and other impurities. Air bubbles were eliminated by keeping the solutions at room temperature for 2 h.

A similar 10 g L^{-1} solution of agar and PVA were prepared by dissolving 5 g in 500 mL preheated ultrapure water**.** The solutions was then stirred and kept at about 90^{o}C for 3 h.

3.2.2 Preparation of the blended films

The preparation of blend films of chitosan and agar or PVA was carried out at various proportions. The aqueous agar or PVA solution was added drop by drop to the chitosan solution, under continuous stirring at 90^{o}C in various proportions by volume. The range of the added agar or PVA to chitosan solution varied from 0-50 vol%. Stirring was allowed to continue for 30 minutes after mixing. Films of the resulting homogeneous solutions were obtained by casting prescribed amounts of the solution onto polystyrene petri dishes followed by drying at 60^{o}C for 48 h. The films were carefully peeled off and kept under evacuated desiccator over fresh silica gel until use. All films obtained were transparent, uniform and free of air bubbles.

Similar films from pure chitosan, pure agar and pure PVA were prepared using the same casting procedure and used as references.

3.2.3 Film thickness

The film thickness was measured with a digital micrometer (Mitutoyo, Japan) with 0.001mm resolution. Several thickness measurements were taken at various positions on each specimen and the average value was determined.

3.3 Methods and equipments

3.3.1 Molecular weight measurements

The molecular weight of chitosan was 5.5 $\times 10^5$ g mol^{-1} determined by gel permeation chromatography (GPC) equipped with a Waters 1515 HPLC Pump and a Waters 2414 Refractive Index Detector. The column used was PL aquagel-OH 30 (8 μm, 300 × 7.5 mm) and the solvent used was 1% acetic acid. The molecular weight of agar was about 1.3 $\times 10^4$ g mol^{-1} using the same method.

3.3.2 Viscosity measurements

The rheological measurements were performed on a Brookfield digital viscometer, model DV-II + Pro, with an attached UL adapter. The viscosity was determined in 20 mL of the sample and the shearing time was 15 seconds. For the storage time measurements, solutions were kept at room temperature in glass bottles in a dark place until analysis. Each measurement was recorded as an average value of five readings when a constant shear rate (6.15 s^{-1}) was applied.

3.3.3 FTIR measurements

The FTIR measurements of blended films (10 μm thick) were performed on a spectrometer (Perkin Elmer, model 2000). The spectra were obtained at a frequency range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1} and 8-times scanning.

3.3.4 Scanning electron microscopy (SEM) measurements

The SEM measurements were carried out to study the surface morphology and to study the compatibility of the blended films. In this work, the surface morphology was investigated by a FEI Quanta 200F field emission scanning electron microscope (FESEM) (FEI, USA) controlled by a 32 bit computer system using Microsoft Windows 2000 as an operating system.

3.3.5 Mechanical properties

Mechanical studies were carried out to study the tensile strength and elongation of the blended films. Mechanical testing plays an important role in evaluating fundamental properties of materials, developing new materials and controlling the quality of materials.

The mechanical properties of the films were measured by a universal mechanical tester (Instron, Model 5566, USA) according to the ASTM Standard Method D 882-91 (ASTM, 1995). Dumbbell-shaped specimens of 50 mm long with a neck of 28 and 4 mm wide (Figure 3.1) were used. The measurements were carried out at 23^{o}C and 50% relative humidity. The crosshead speed was fixed at 50 mm/min. A minimum of five specimens were tested for each sample and the average was obtained.

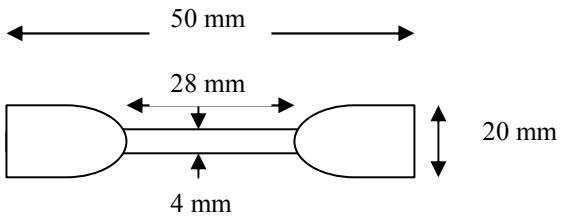


Figure 3.1. A schematic diagram of a specimen

3.3.6 Thermal studies

Thermal analysis provides valuable information regarding toughness, stability and miscibility in blends. In this work, two techniques have been employed as follows:

Thermogravimetric measurements (TGA)

In this work, TGA was used to evaluate the thermal stability and to determine the decomposition temperature of chitosan and its blends. Thermogravimetric measurements were performed using a Mettler-Toledo thermogravimetric analyzer model TGA/SDTA851e. TGA runs were carried out in a temperature range of 30-500^{o}C, under nitrogen atmosphere, and with a constant heating rate of 10^{o}C per minute in all the experiments. The sample size of 4-10 mg was weighed and the mass of the sample pan was continuously recorded as a function of temperature.

Differential scanning calorimetry measurements (DSC)

DSC studies were performed using a DSC Mettler Toledo (model. DSC822e). The samples were scanned under a nitrogen atmosphere at a constant rate of 10^{o}C/min. The experiment consisted of two runs. The first run was performed in heating range of 40^{o}C to 500^{o}C and the second one was from 40^{o}C to 350^{o}C, after heating up to 200^{o}C and cooling to 40^{o}C to eliminate the effect of moisture.

3.3.7 Degree of swelling

The swelling behavior of the films was measured by immersing the blend films in distilled water at room temperature for 10 h. The excess water on the surface of the films was removed by blotting the surface with tissue paper, and the weight was then recorded. This

process was repeated at least three times for each sample. The degree of swelling was calculated using the following equation:

$$\text{Degree of swelling (\%)} = [(W_2-W_1)/ W_1]x\ 100$$

Where, W_1 is the weight of completely dried sample and W_2 is the weight of swelled sample.

3.3.8 Contact angle measurements

The static water contact angles of the films were measured at room temperature by the drop method using an optical contact angle meter CAM 200 (KSV Instruments Ltd, Helsinki**,** Finland) to examine the surface wettability of the films. The substrates used for the experiments were glass microscope slides (25.4 × 76.2 mm, 1-1.2 mm thick). Each slide was cleaned before use by soaking in ethanol overnight. 7 μ L of distilled water was carefully injected on the film surface before measuring and the measurement time was 24 second. The contact angles were measured on both sides of the drop and averaged. Each reported contact angle was the mean value of at least 10 measurements.

4.0 Chapter 4: Results and discussion (rheology of the blended solutions)

4.1 General introduction

The results and discussion is divided into four chapters: (i) Chapter 4 deals with the rheology of the three types of polymer blended solutions (CS/AG, CS/PVA and CS/AG/PVA). It also includes the investigation of some parameters affecting the rheology of these blended solutions and the calculation of the viscous flow activation energies for the blend concentration to study the compatibility of the polymers (ii) Chapter 5: The blended films of CS/AG are characterized by employing FTIR, SEM, thermal studies, mechanical tests, swelling test together with the contact angle measurements and the obtained results are interpreted and analyzed (iii) Chapter 6 which includes the characterization of blending of natural and synthetic polymers (CS/PVA) using the same techniques mentioned in chapter 5 and (iv) Chapter 7 in which the ternary blended films of the three polymers (CS/AG/ PVA) with various blend ratios are characterized by employing the same techniques mentioned in chapter 5 and 6.

4.2 Rheological study of chitosan blended solutions

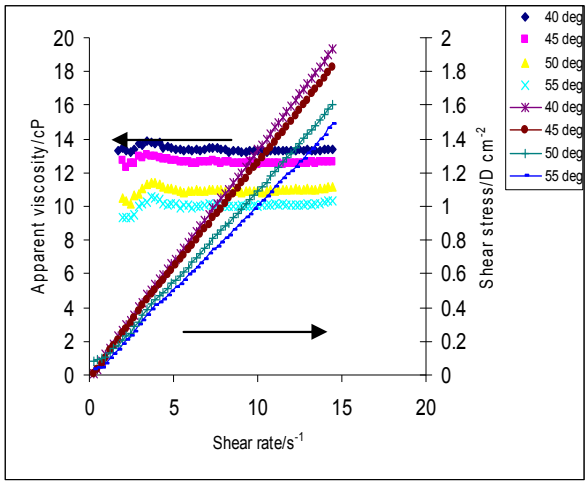
4.2.1 Rheological study of chitosan/agar blended solutions

The effect of temperature on the shear viscosity of chitosan/agar blended solutions.

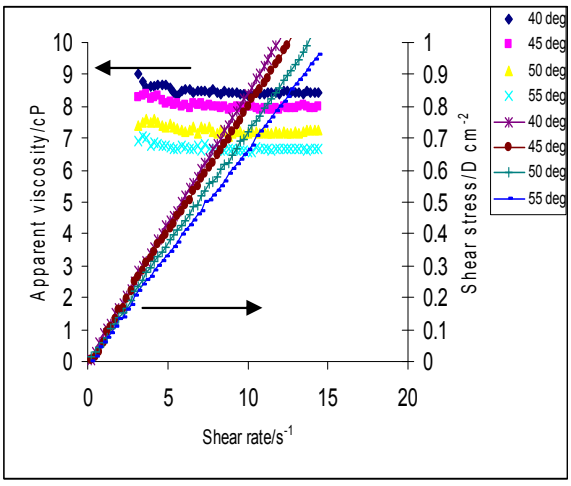
In this study, the shear rate-dependent viscosity of CS/AG blended solutions as a function of shear rate at various temperatures in the range of 45 to 55^{o}C is shown in Figure 4.1(a-g). Higher temperatures were not applied in order to avoid thermal degradation of the polymers and solvent evaporation while lower temperatures could not be applied due to the gel formation in some CS/AG blended solutions. The behavior of the viscosity–temperature interrelationship for these blends showed a decrease in the viscosity with an increase in the

temperature, which is consistent with many polymer solutions. Also, a Newtonian behavior (in which the relation between the shear stress or apparent viscosity and the shear rate is linear) was observed at all temperatures for the ratios 100/0, 90/10, 80/20 and 70/30. However, the increase in shearing viscosity and the appearance of shear thinning behavior were clearly noticed for the ratios of 60/40 and 50/50. It is expected that the increase in viscosity for CS/AG blends and the appearance of a clear shear thinning behavior is attributed to the formation of hydrogen bonding due to the interaction among the functional groups of CS and AG (–OH and $–NH_2$ groups in CS and –OH groups in AG).

Figure 4.1 also show the effect of temperature on the shear stress of CS/AG blended solutions as a function of shear rate. Shear stress increased with increasing shear rate for all ratios. At the same shear rate, shear stresses were higher at lower temperatures. In addition, the increases in shear stress with increasing shear rate were found to be more remarkable at lower temperatures. From the graph it is clear that only the ratios 60/40 and 50/50 exhibit pseudoplastic non-Newtonian behavior (where the rate of shear is not proportional to the corresponding shear stress or apparent viscosity). The results of pure CS solution obtained are in good agreement with previous studies (Desbrieres, 2002). In addition, these figures indicate that the temperature has more effect on the solutions at lower shear rate values, *i.e*., the viscosity increase associated with temperature is less important at high shear rates. Similar behavior was also reported in the literature for pure CS solutions (Delben *et al*, 1990).


Apparent viscosity/cP
Shear stress/D cm-2
Shear rate/s-1
40 deg
45 deg
50 deg
55 deg

(a)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Shear stress/D cm-2
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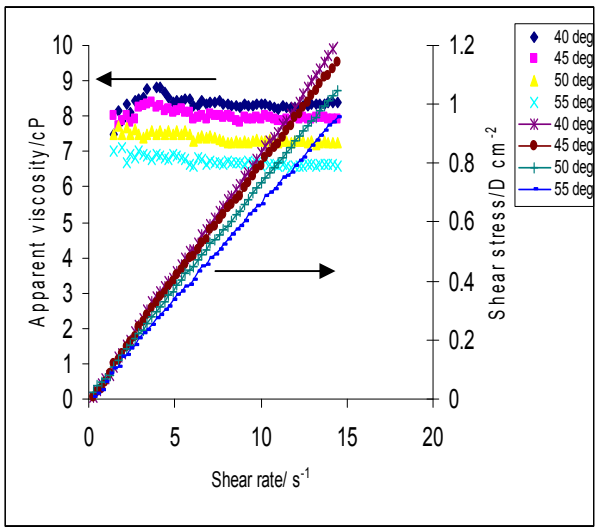
(b)


Apparent viscosity/cP
Shear stress/D cm-2
Shear rate/ s-1
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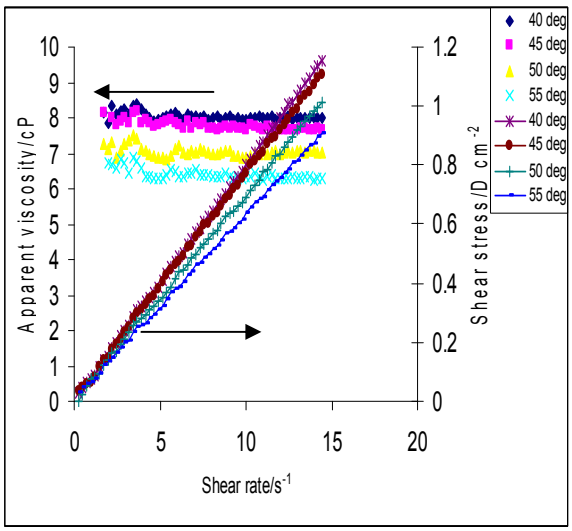
55 deg

(c)


Apparent viscosity/cP
Shear stress/D cm-2
Shear rate/s-1
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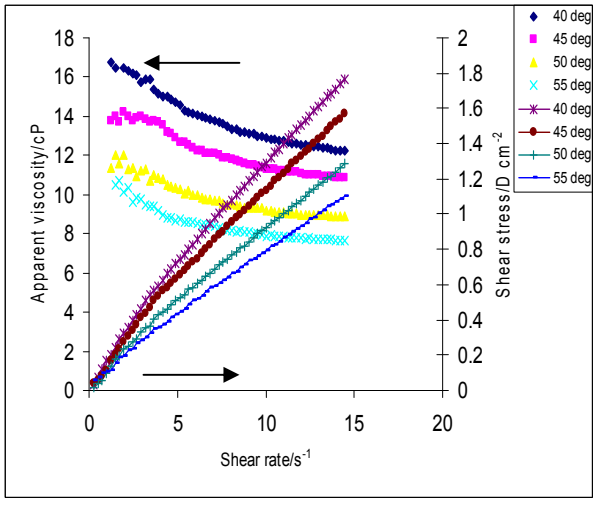
(d)


Apparent viscosity/cP
Shear stress/D cm-2
Shear rate/s-1
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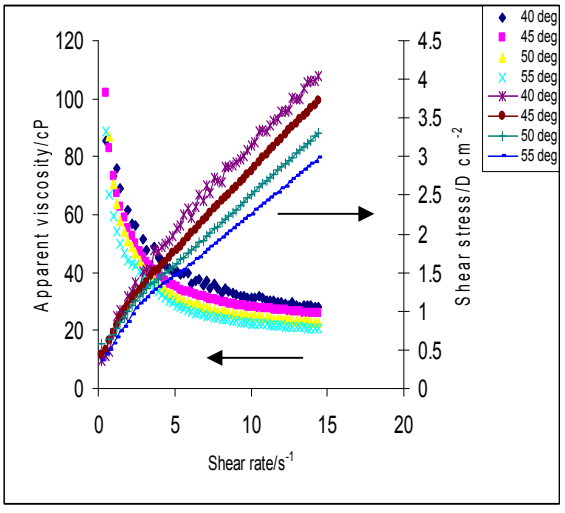
(e)


Apparent viscosity/cP
Shear stress/D cm-2
Shear rate/s-1
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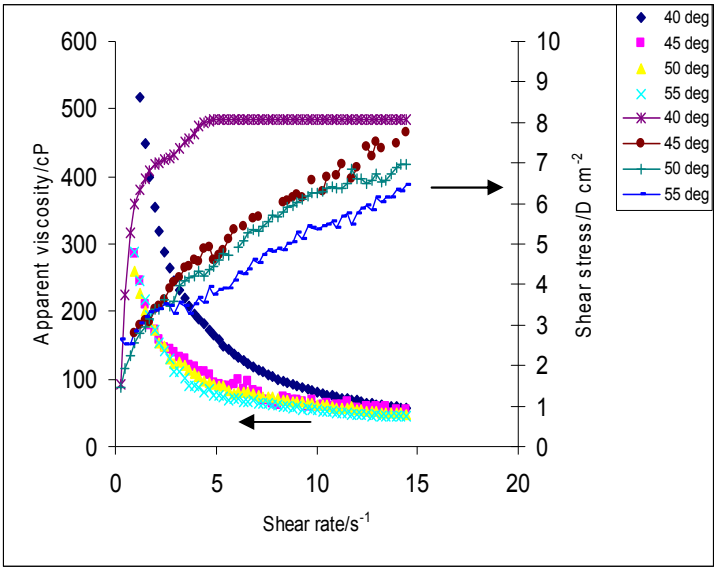
45 deg
50 deg
55 deg

(f)



(g)

Figure 4.1: The apparent viscosity and shear stress versus shear rate at various temperatures of: (a) pure CS, (g) pure AG and their blended solutions with various proportions: (b) (90/10), (c) 80/20, (d) 70/30, (e) 60/40 and (f) 50/50.

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The relationship between apparent viscosity (determined at a constant shear rate) and temperature for CS/AG blends and the pure components is demonstrated in Figure 4.2. Linear relationships can be observed (Table 4.1) whereby the viscosity of the blended solutions decreases with increasing temperature, i.e., it is a strong function of temperature. It can also be noted that the most effected solution by temperature is the pure agar solution followed by the ratio of 50/50. Detailed equations of the viscosity-temperature correlations are given in the appendix.

The viscosity values obtained at a constant shear rate can be correlated with temperature according to the Arrhenius equation:

$$\eta = A \,.\, e^{-Ea/RT} \qquad (4.1)$$

where A is a constant related to molecular motion, E_a is the activation energy for viscous flow at a constant shear rate, R is the gas constant and T is the absolute temperature in K. A plot of ln viscosity versus reciprocal of temperature ($1/T$) should produce a straight line and from its slope the E_a was calculated. Knowing the activation energy is important in deducing the sensitivity of a process towards temperature (Turhan and Gunasekaran, 2002). The higher the activation energy, the more sensitive is the process to temperature. The activation energy is also useful for signifying the minimum amount of energy required for the reactants to start a reaction (Fogler, 1999). The lower the activation energy, the faster the reaction will proceed. Figure 4.3 shows the Arrhenius plots for various proportions of CS/AG blended solutions. Linear relationships can be observed. The values of the apparent activation energy are presented in Table 4.2.

The value of the activation energy of pure CS is in accordance with the reported values. For example, Wang and Xu (1994) reported that the activation energy varies from 25 kJ mol^{-1} when DD is 91% to 15 kJ mol^{-1} when DD is 75% for a chitosan concentration of 20 g L^{-1} (in 0.2 M AcOH/0.1 M AcONa). Desbrieres (2002) reported values of activation energy at zero shear rate vary from about 15 to 37 kJ mol^{-1} for a chitosan concentration in the range 0 - 40 g L^{-1} (solvent, 0.3 M AcOH/0.05 M AcONa).

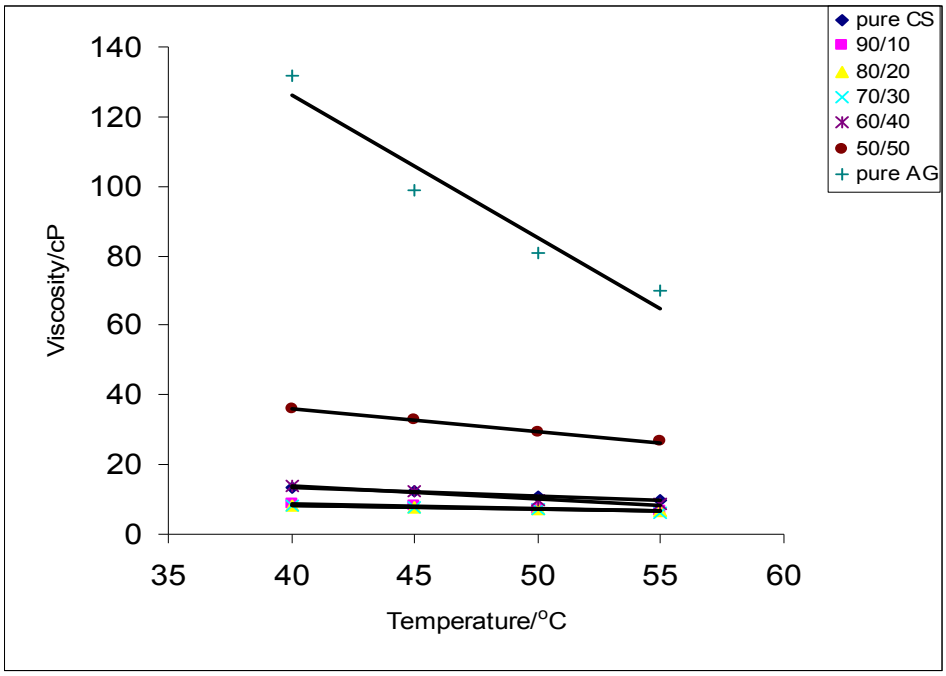


Figure 4.2: The relationship between the apparent viscosity and the temperature of the pure CS, pure AG and their blended solutions having various ratios at a constant shear rate.

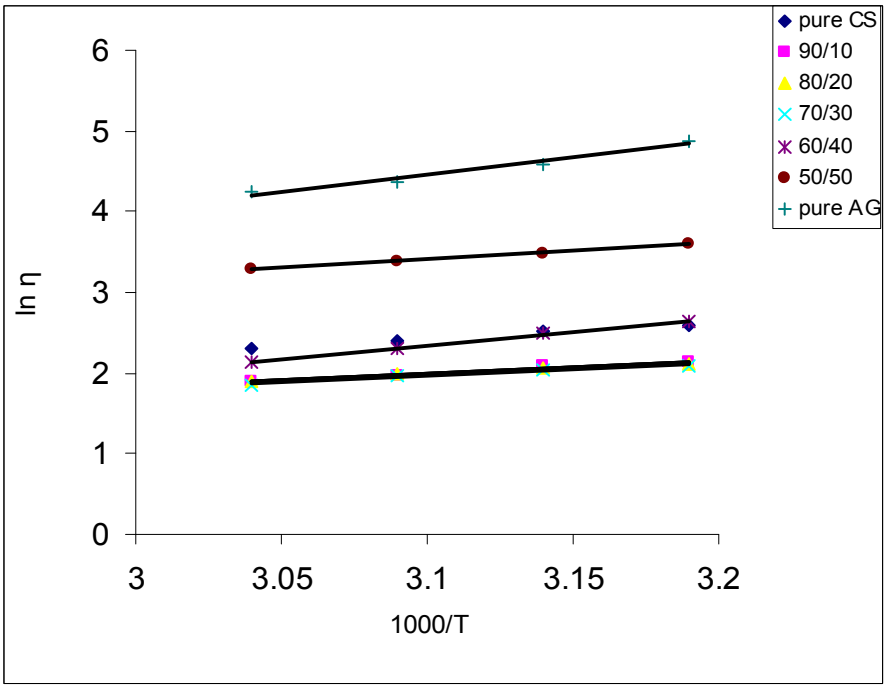


Figure 4.3: The Arrhenius plot of ln η versus 1/T for the pure CS, pure AG and their blended solutions having various ratios.

Table 4.1: Relationship between the R^2 and the blend concentration.

Blend ratio (CS/AG)	R^2
100/0	0.9821
90/10	0.9869
80/20	0.9796
70/30	0.9567
60/40	0.9934
50/50	0.9972
0/100	0.9398

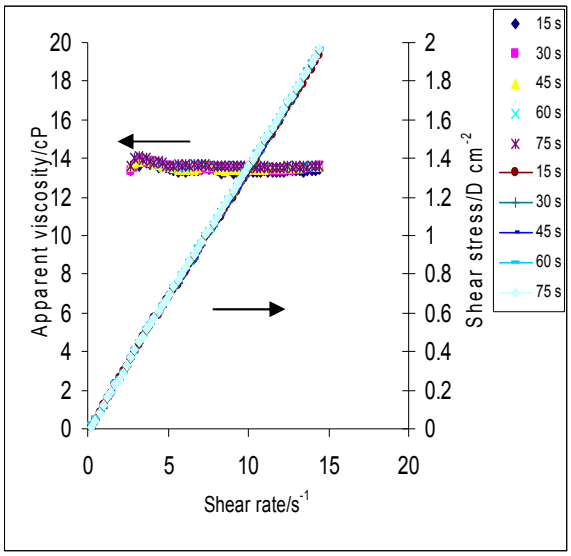
Table 4.2: The values of the activation energy of CS, AG and their blended solutions at different ratios.

Blend ratio CS/AG	Viscous flow activation energy, E_a (kJ mol^{-1}) at 6.15 s^{-1}
100/0	16.79
90/10	13.30
80/20	12.30
70/30	12.97
60/40	28.27
50/50	17.29
0/100	35.41

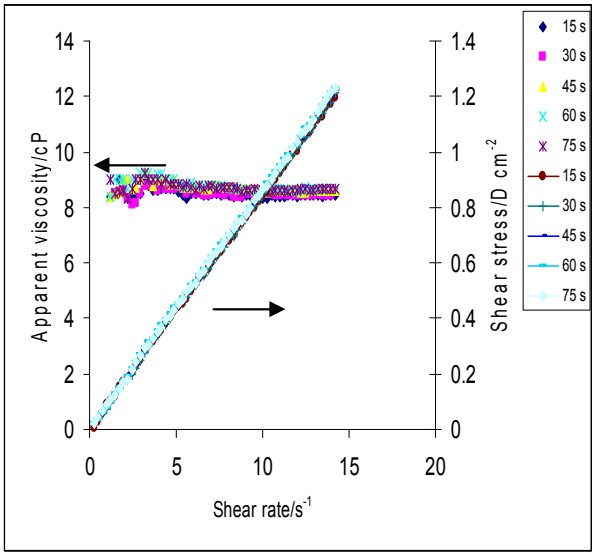
The effects of shearing and storage time on the apparent viscosity of CS/AG blended solutions.

Viscosity measurements were performed as a function of shear rate for time intervals of 15, 30, 45, 60 and 75 seconds at 40^{o}C to study the shearing time effect as shown in Figure 4.4 (a-g) (it was difficult to study the effect of shearing time for the pure AG due to its random behavior at this temperature). At all shearing times, CS/AG blended solutions exhibited similar behavior and no significant change was observed with the exception of the ratio 50/50 and the pure AG solution whereby increasing in shearing time led to a decrease in the shearing viscosity and shear stress in general. In addition, different behaviors were

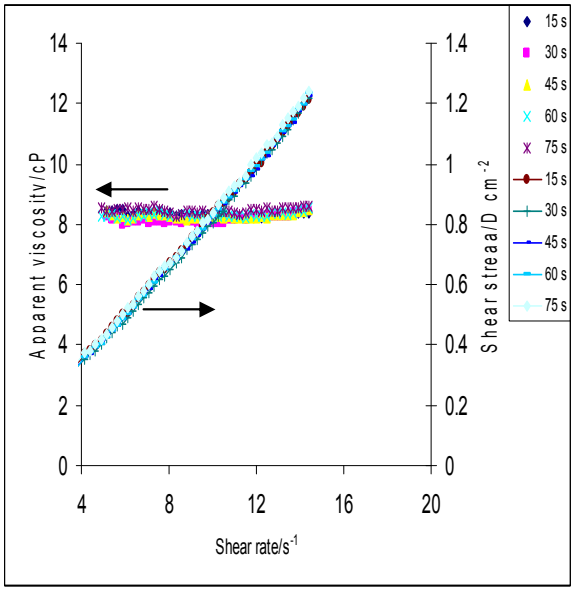
observed for the blended solutions when the period of storage was extended to three weeks at a constant shear rate (Figure 4.5). Pure CS showed gradual increase in the viscosity while a drop in viscosity from about 130 to 35 cP was recorded within the first week for pure agar, after which it decreased gradually. However, all CS/AG blended solutions recorded a big increase in viscosity within the first and second weeks while a different trend was observed in the third week. The rapid increase in viscosity with time may indicate that some strong interactions have taken place among the chains of the two polymers.



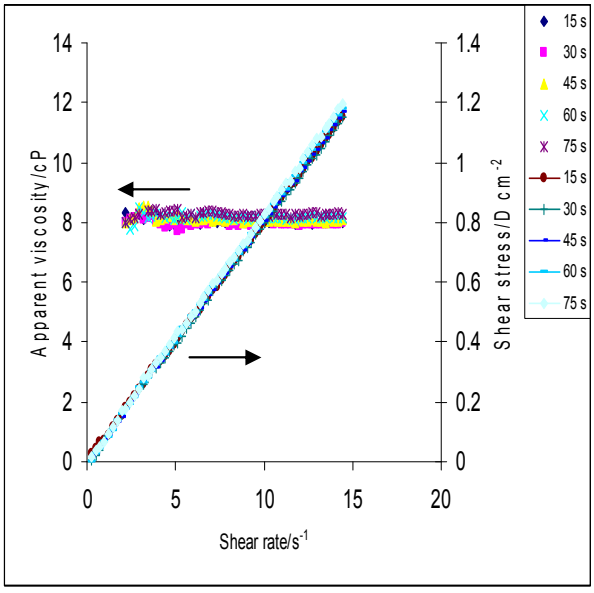
(a)



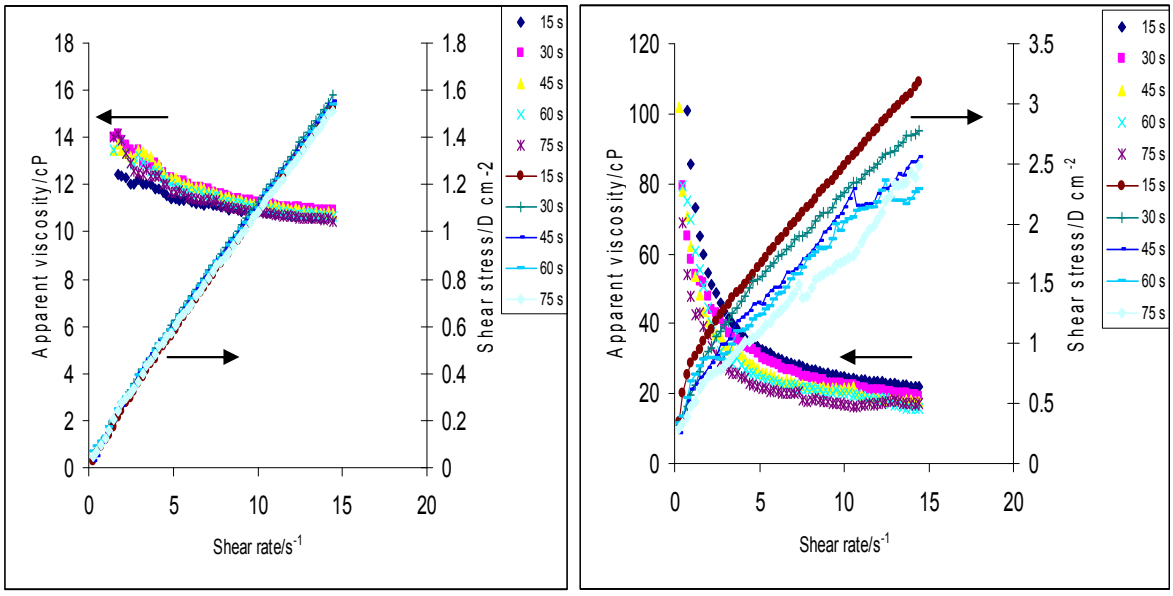
(b)



(c)



(d)



(e) (f)

Figure 4.4: The apparent viscosity and shear stress versus shear rate at various shearing times of: (a) pure CS and CS/AG blended solutions with various proportions: (b) (90/10), (c) 80/20, (d) 70/30, (e) 60/40 and (f) 50/50 at 40°C.

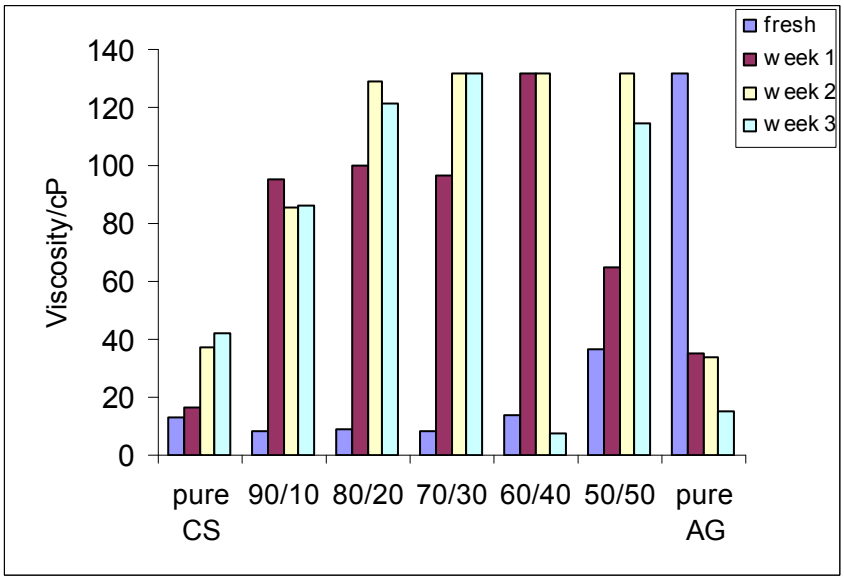
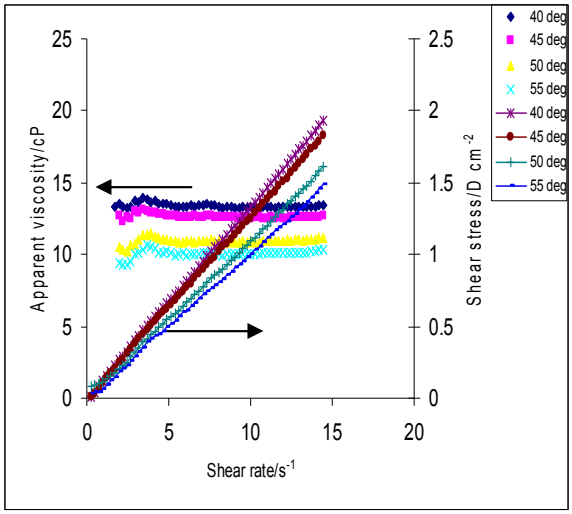


Figure 4.5: The effect of storage time on the apparent viscosity (determined at a constant shear rate) of pure CS, pure AG and their blended solutions (SD is very small).

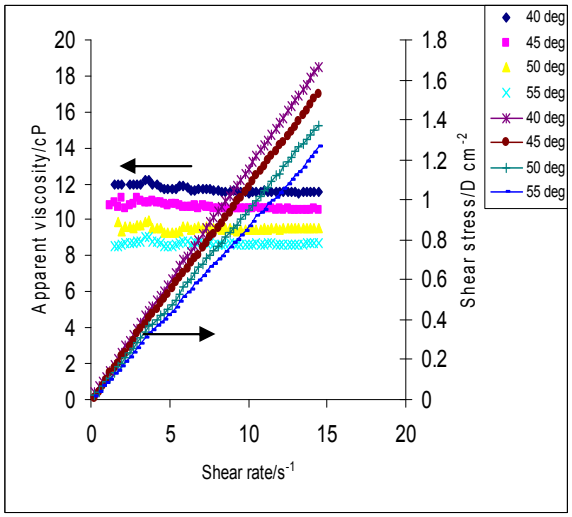
4.2.2 Rheological study of chitosan/PVA blended solutions

The effect of temperature on the shear viscosity of chitosan/PVA blended solutions.

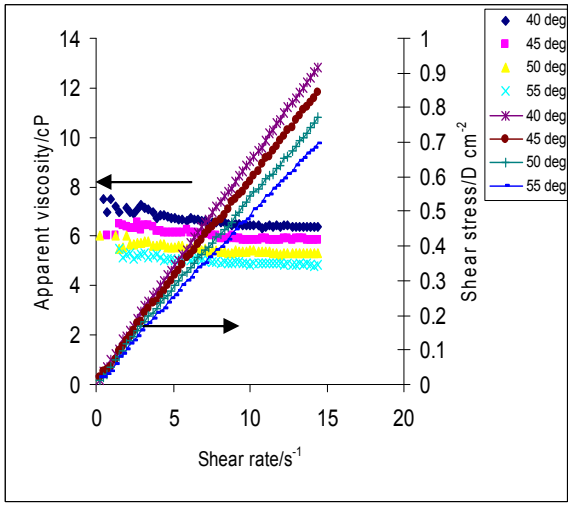
The shear rate-dependent viscosity of CS/PVA blended solutions as a function of the shear rate at a temperature range of 45 to 55^{o}C is presented in Figure 4.6 (a-g). Similar to that of CS/AG blended solutions, the behavior of the viscosity–temperature interrelationship for these mixtures show a decrease in the viscosity with the increase in the temperature. However, the Newtonian behavior was observed at all temperatures studied for all ratios. These results do not suggest any kind of interaction between the two components. Figure 4.6 also shows the effect of temperature on the shear stress of CS/PVA blended solutions as a function of shear rate. The shear stress increased with increasing shear rate for all ratios. At the same shear rate, shear stresses were higher at lower temperatures. In addition, an increase in shear stress with increasing shear rate was found to be more significant with the gradual decrease in the temperature within the investigated range. From the graph it is clear that all of the solutions exhibit Newtonian behavior at the mentioned range of temperature. This figure also indicates that the temperature effect is more profound in the solutions at lower shear rate values.


Apparent viscosity/cP
Shear stress/D cm^{-2}
Shear rate/s^{-1}
40 deg
45 deg
50 deg
55 deg

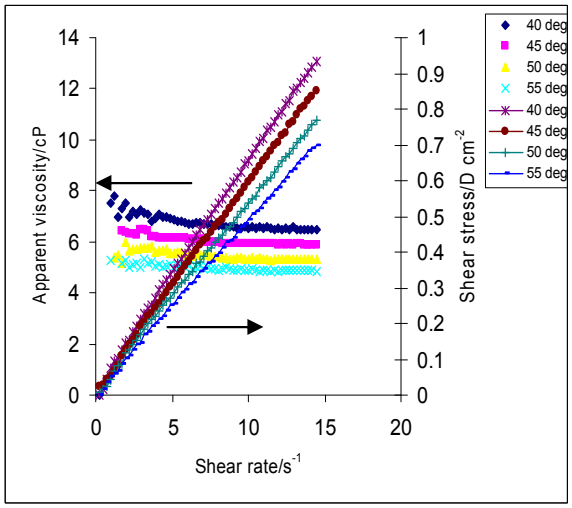
(a)


Apparent viscosity/cP
Shear stress/D cm^{-2}
Shear rate/s^{-1}
40 deg
45 deg
50 deg
55 deg

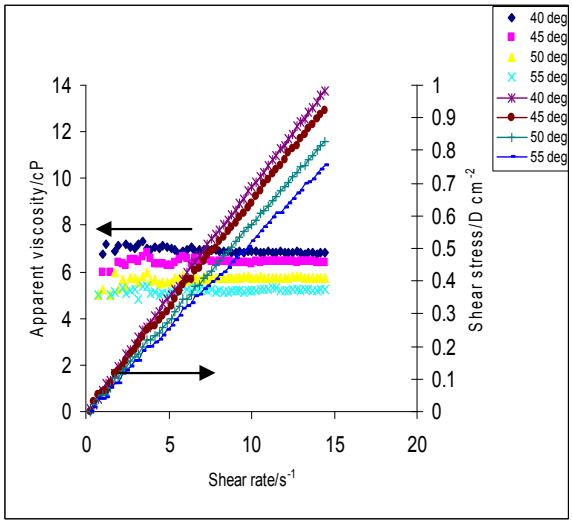
(b)


Apparent viscosity/cP
Shear stress/D cm^{-2}
Shear rate/s^{-1}
40 deg
45 deg
50 deg
55 deg

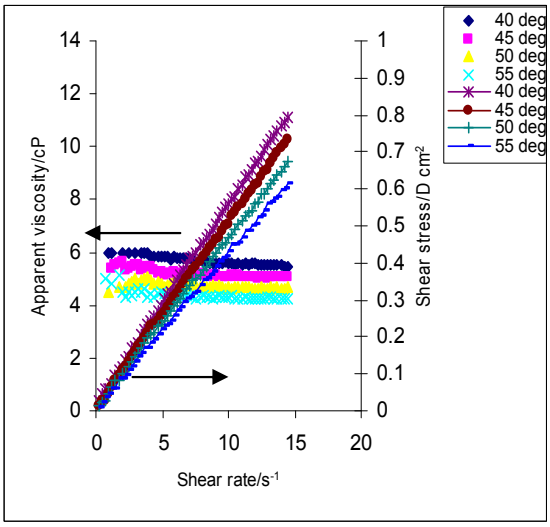
(c)


Apparent viscosity/cP
Shear stress/D cm^{-2}
Shear rate/s^{-1}
40 deg
45 deg
50 deg
55 deg

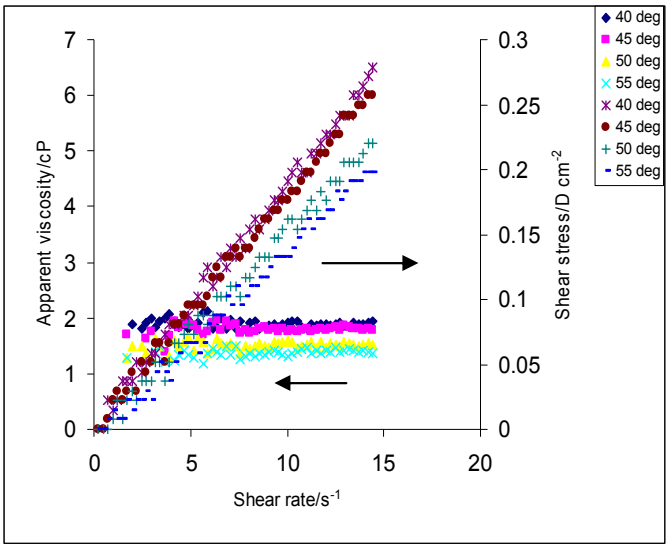
(d)


Apparent viscosity/cP
Shear stress/D cm^{-2}
Shear rate/s^{-1}
40 deg
45 deg
50 deg
55 deg

(e)


Apparent viscosity/cP
Shear stress/D cm^{2}
Shear rate/s^{-1}
40 deg
45 deg
50 deg
55 deg

(f)



(g)

Figure 4.6: The apparent viscosity and shear stress versus shear rate at various temperatures of: (a) pure CS, (g) pure PVA and their blended solutions with various proportions: (b) (90/10), (c) 80/20, (d) 70/30, (e) 60/40 and (f) 50/50.

The relationship between shear viscosity (determined at a constant shear rate) and temperature for CS/PVA blended solutions and their pure components is demonstrated in Figure 4.7. This plot shows linear relationships (Table 4.3) whereby the viscosity of the blended solutions decreases with increasing temperature, i.e., it is a strong function of temperature. It can also be noted that the most affected solution by the temperature, with respect to viscosity, is the pure CS solution while the lowest affected one is the pure PVA solution. This indicates that increasing PVA concentration mostly reduces the sensitivity of the obtained blended solutions towards temperature.

The viscosity values of the CS/PVA blended solutions obtained at a constant shear rate can be also correlated with temperature according to the Arrhenius equation. Figure 4.8 shows the Arrhenius plot for various proportions of CS/PVA blended solutions. This graph also

shows linear relationships between η and 1/T. The values of the apparent activation energy are shown in Table 4.4. As it can be seen in Table 4.4, all the blended solutions have lower values of E_a comparing to the pure components, which reflects high possibility of compatibility between the two polymers.

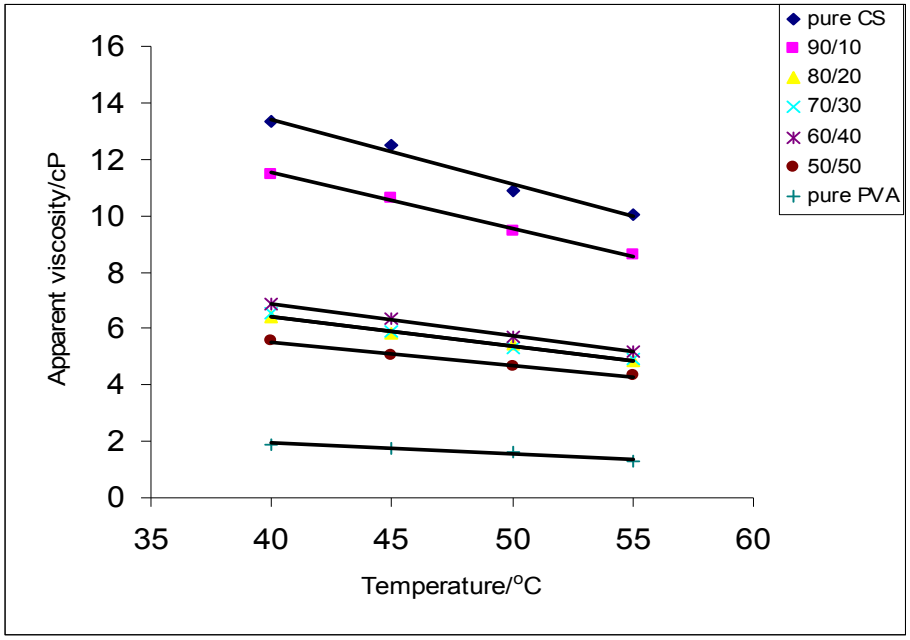


Figure 4.7: The relationship between the apparent viscosity and the temperature of the pure CS, pure PVA and their blended solutions at various ratios at a constant shear rate.

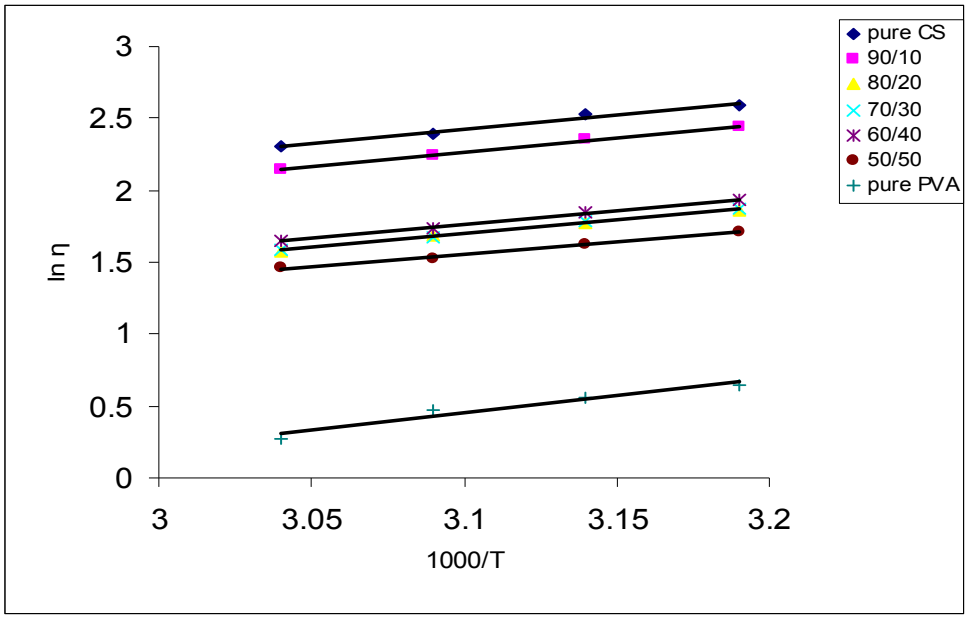


Figure 4.8: The Arrhenius plot of ln η versus 1/T for the pure CS, pure PVA and their blended solutions having various ratios.

Table 4.3: Relationship between the R^2 and the blend concentration.

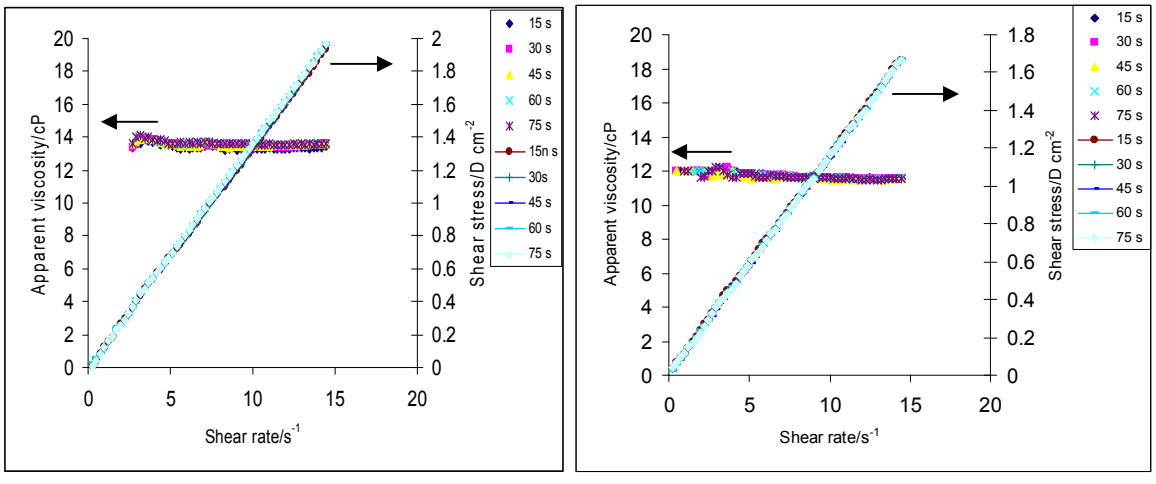
Blend ratio (CS/PVA)	R^2
100/0	0.9821
90/10	0.9966
80/20	0.9973
70/30	0.9973
60/40	0.9615
50/50	0.9818
0/100	0.8824

Table 4.4: Values of the activation energy of chitosan, PVA and their blended solutions at different ratios.

Blend ratio (CS/PVA)	Viscous flow activation energy, E_a (kJ mol^{-1}) at 6.15 s^{-1}
100/0	16.80
90/10	16.30
80/20	15.63
70/30	15.63
60/40	16.63
50/50	14.13
0/100	19.29

The effects of shearing and storage time on the apparent viscosity of CS/PVA blended solutions.

The viscosity measurements were performed as a function of shear rate for 15, 30, 45, 60 and 75 seconds at 40^{o}C to study the shearing time effect (Figure 4.9). At all shearing times, CS/PVA blended solutions exhibit similar behavior and no significant change was observed. In addition, when the period of storage was extended to three weeks at a constant shear rate, a general decrease in viscosity was observed as the PVA content increases (Figure 4.10). Pure chitosan showed gradual increase in the viscosity as a function of time while almost a constant value of viscosity was recorded for pure PVA. However, all CS/PVA blended solutions recorded a decrease in the viscosity as the period of time was extended to three weeks in general. These results suggest the absence of any interaction between the two polymers in the mixtures. This could be due to the chitosan dominating the blends with PVA making them to behave similar to pure chitosan. This is attributed to the bulk molecular structure with high viscosity of CS compared to the unbranched linear structure with low viscosity of PVA.



(a) (b)

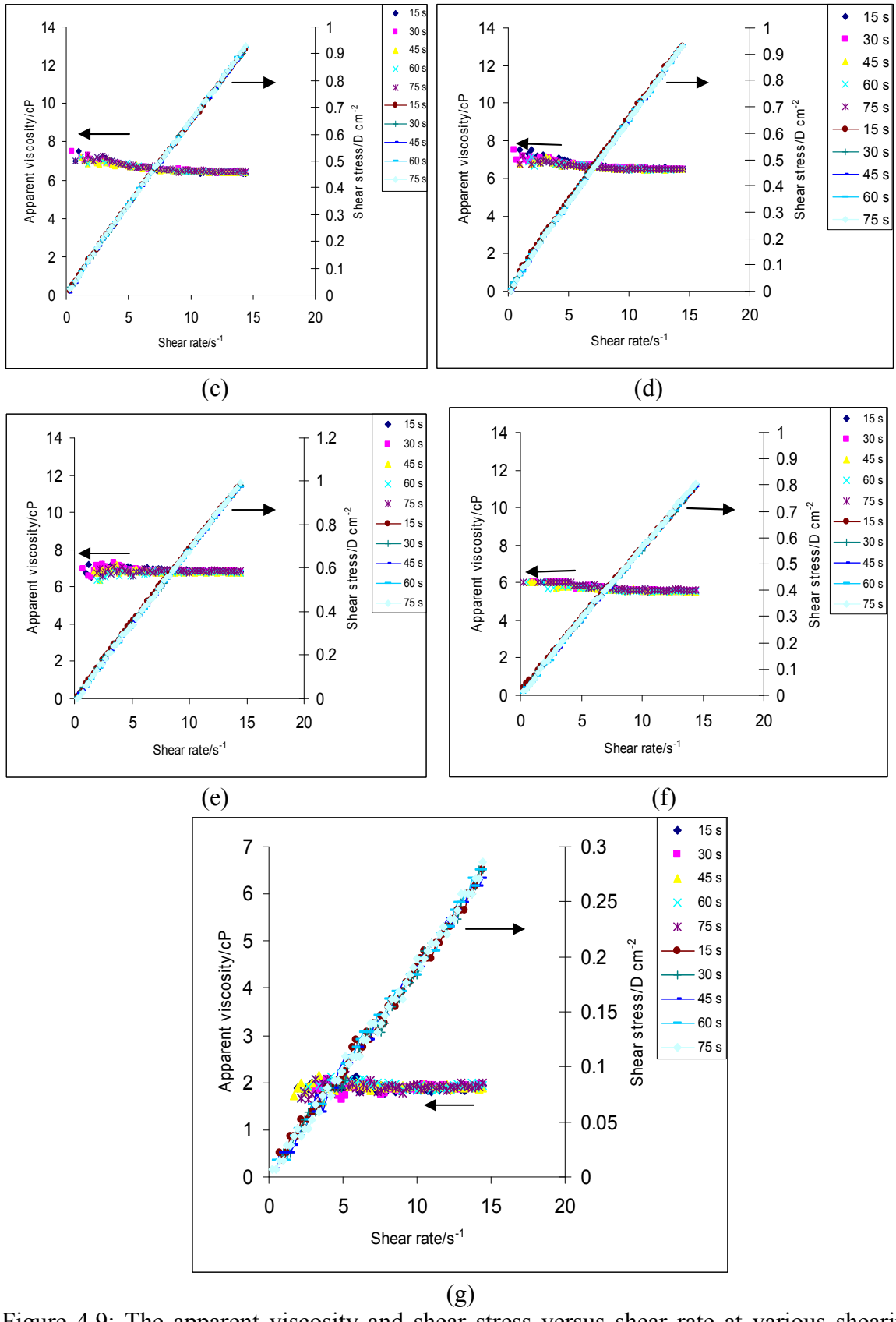


Figure 4.9: The apparent viscosity and shear stress versus shear rate at various shearing times of: (a) pure CS, (g) pure PVA and their blended solutions with various proportions: (b) (90/10), (c) 80/20, (d) 70/30, (e) 60/40 and (f) 50/50 at 40^{o}C.

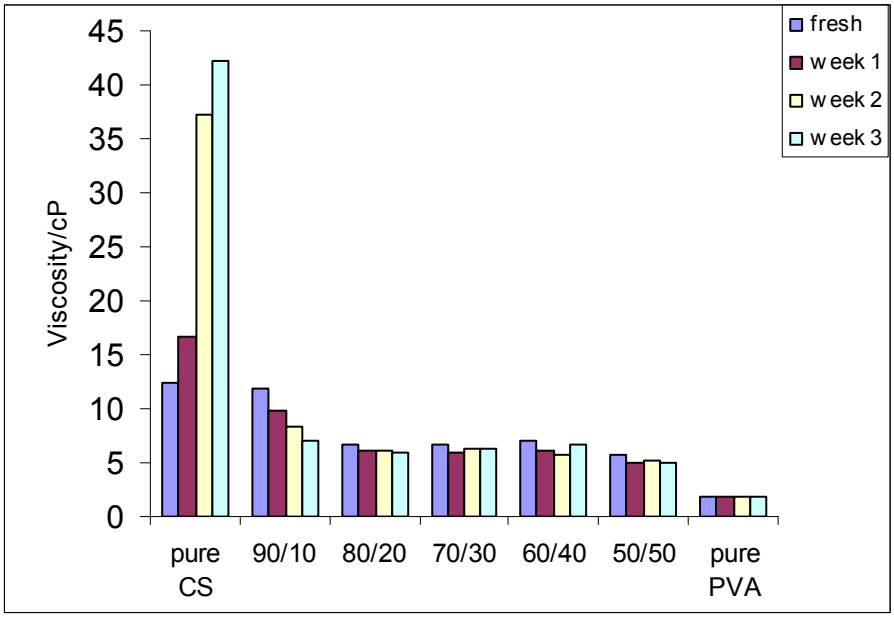


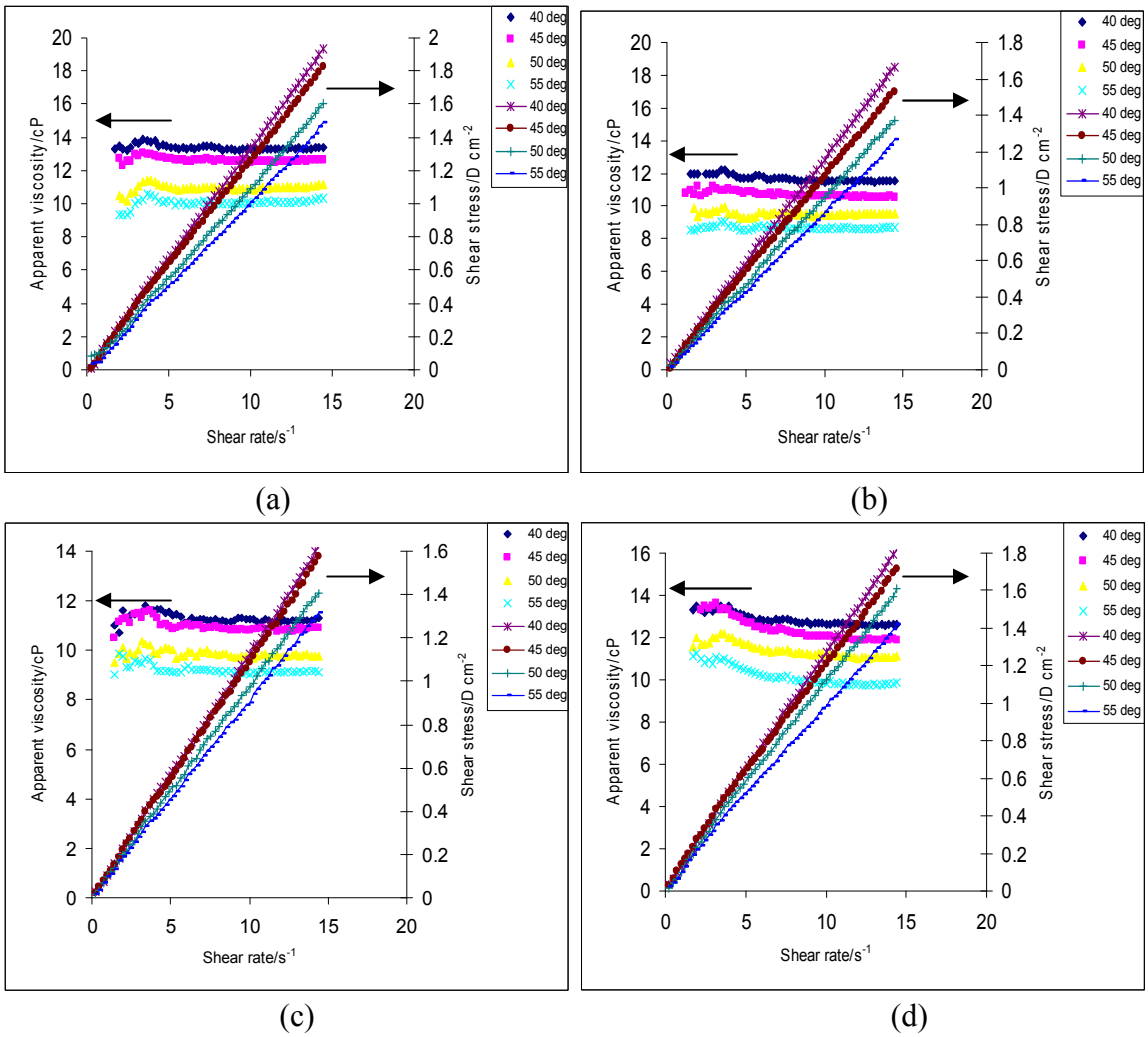
Figure 4.10: The effect of storage time on the shearing viscosity (determined at a constant shear rate) of pure CS, pure PVA and their blended solutions (SD is very small).

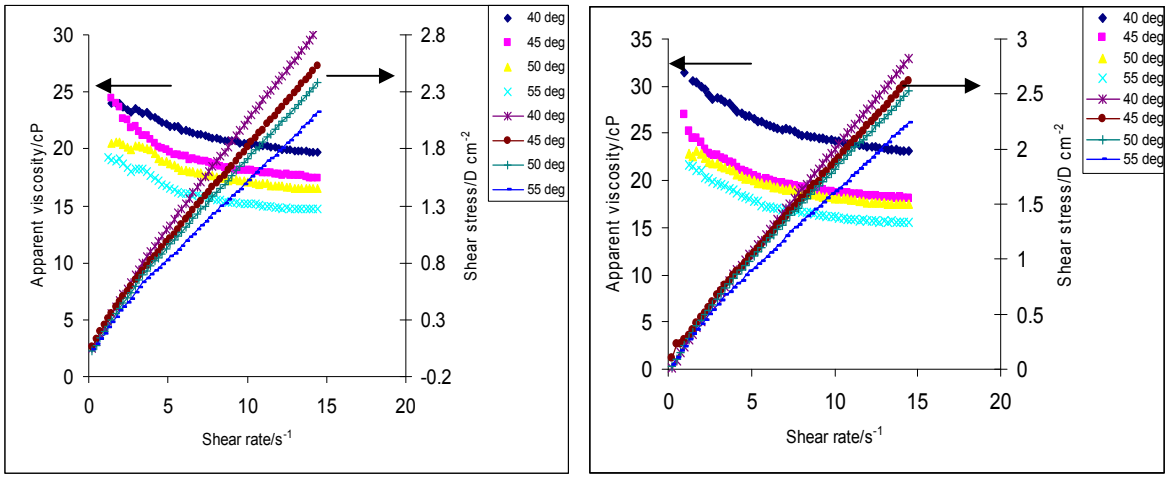
4.2.3 Rheological study of chitosan/agar/ polyvinyl alcohol ternary blended solutions

The effects of temperature on the shear viscosity of CS/AG/PVA ternary blended solutions.

The shear rate-dependent viscosity of CS/AG/PVA blended solutions as a function of shear rate at a temperature range of 45^{o}C to 55^{o}C is presented in Figure 4.11. The Newtonian behavior is observed at all temperatures for the ratios 90/05/05 and 80/10/10. However, the increase in shearing viscosity and the appearance of shear thinning behavior begin to appear at the ratio 70/15/15 and it is more clearly at higher compositions of agar and PVA. It is expected that the increase in viscosity for CS/AG/PVA blends is attributed to the formation of hydrogen bonding due to the interaction among the functional groups of CS, AG and PVA (–OH and –NH_2 groups in CS, –OH groups in AG and –OH groups in PVA).

The effect of temperature on the shear stress of CS/AG/PVA blend solutions as a function of shear rate is also shown in Figure 4.11. There is an increase in shear stress with increasing shear rate for all ratios. At the same shear rate, shear stresses were higher at lower temperatures. In addition, it is clear from the graph that only the proportions 70/15/15, 60/20/20 and 50/25/25 exhibit pseudoplastic non-Newtonian behavior.





(e) (f)

Figure 4.11: The apparent viscosity and shear stress versus shear rate at various temperatures of: (a) pure CS and its blended solutions with AG and PVA at various proportions: (b) (90/05/05), (c) 80/10/10, (d) 70/15/15, (e) 60/20/20 and (f) 50/25/25.

The relationship between the shearing viscosity (determined at a constant shear rate) and temperature for CS/AG/PVA ternary blended solutions is demonstrated in Figure 4.12. This graph shows a nearly linear relationship (Table 4.5) whereby viscosity of the blended solutions decreases with increasing temperature, i.e., it is a strong function of temperature. It can also be noted that the most effected ratios by temperature are 6/20/20 and 50/25/25.

The variation of viscosity dependent on temperature at a constant shear rate can be evaluated according to the Arrhenius equation. Figure 4.13 presents the Arrhenius plot for various proportions of CS/AG/PVA solution mixtures. This graph also shows linear relationships. The values of the apparent activation energy are shown in Table 4.6. As the concentrations of agar and PVA were increased, the activation energy was decreasing until the ratio of 70/15/15, after which the activation energy started increasing to reach 20.8 kJ mol^{-1} for the ratio 50/25/25.

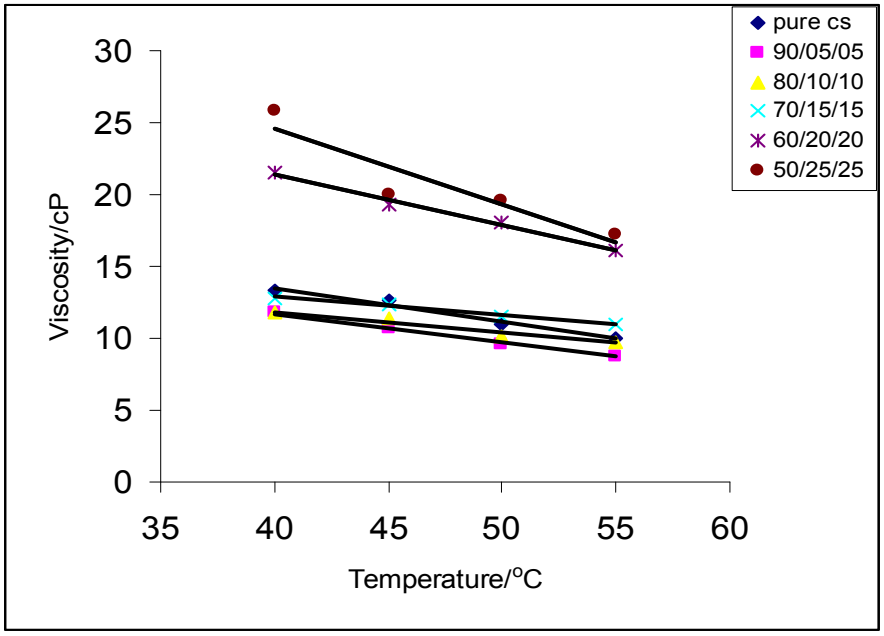


Figure 4.12: The relationship between the apparent viscosity and the temperature of Pure CS and CS/AG/PVA ternary blended solutions having various ratios at a constant shear rate.

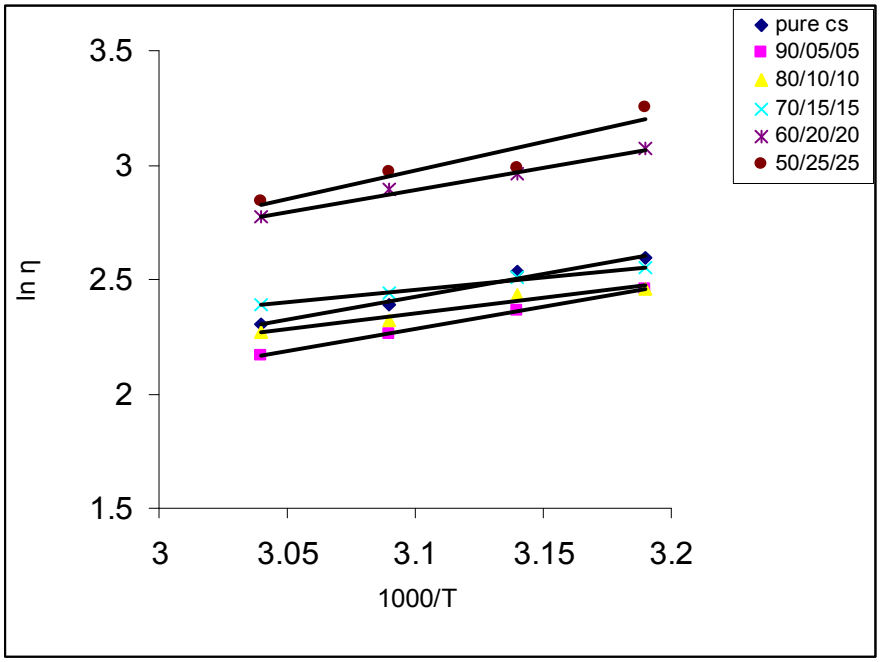


Figure 4.13: The Arrhenius plot of ln η versus 1/T for pure CS and CS/AG/PVA ternary blended solutions having various ratios.

Table 4.5: The relationship between the relation coefficient (R^2) and the blend concentration.

Blend ratio CS/AG/PVA	R^2
100/0/0	0.9821
90/05/05	0.9964
80/10/10	0.9553
70/15/15	0.9912
60/20/20	0.9873
50/25/25	0.8648

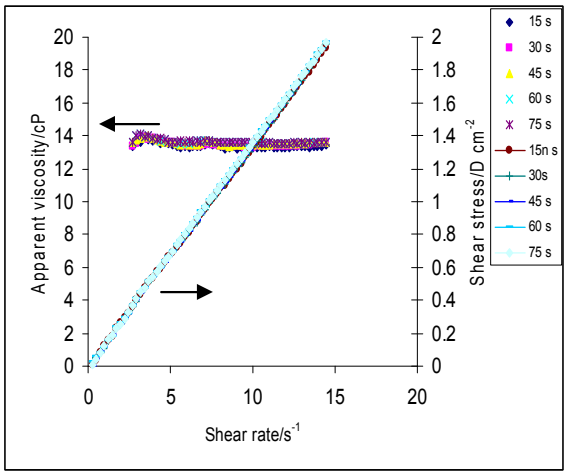
Table 4.6: The values of the activation energy of chitosan and its blended solutions at different ratios.

Blend ratio (CS/AG/PVA)	Viscous flow activation energy, E_a (kJ mol^{-1}) at 6.15 s^{-1}
100/0/0	16.80
90/05/05	16.13
80/10/10	11.31
70/15/15	9.15
60/20/20	16.13
50/25/25	20.80

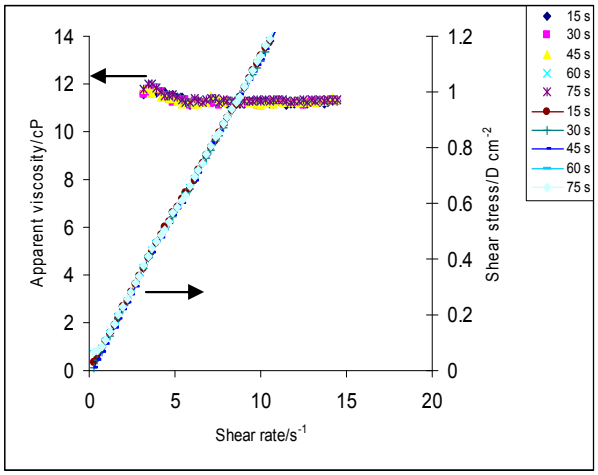
The effects of shearing and storage time on the apparent viscosity of CS/AG/PVA ternary blended solutions.

The effect of shearing time on the apparent viscosity was studied using various amounts of the components for 15, 30, 45, 60 and 75 seconds at 40^{o}C in order to study the shearing time effect (Figure 4.14). For the ratios 90/05/05 and 80/10/10, no significant changes were observed and the apparent viscosity remained almost constant at all shearing times applied. However, the ratios 70/15/15 and 60/20/20 exhibited increasing in the apparent viscosity with increasing shearing time in general, and the solutions became pseudoplastic. This behavior is more obvious for the proportion 50/25/25. Fluids that exhibit increasing in the apparent viscosity with increasing shearing time at a constant shear rate are known as rheopectic (Al-Asheh *et al*, 2002). In addition, different behaviors were observed for the

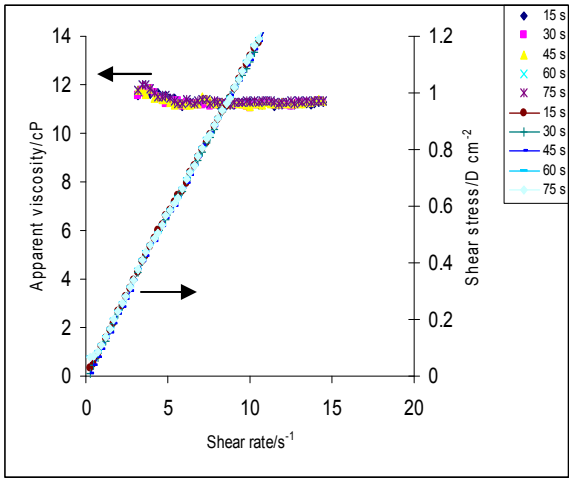
ternary blended solutions when the period of storage was extended to three weeks at a constant shear rate (Figure 4.15). The pure CS solution showed gradual increase in viscosity with time while the proportions 90/05/05 80/10/10, 70/15/15 and 60/20/20 recorded a drastically increase in viscosity within the first week and different in the second and third weeks. However, the blended ratio 50/25/25 recorded the highest value of viscosity (132cP) within the first week, after which the viscosity dropped into about 45cP. This may suggest that the solutions should be used within the first week before starting losing their viscosity. The rapid increase in viscosity with time may indicate that some strong interactions have taken place among the chains of the three polymers.



(a)



(b)



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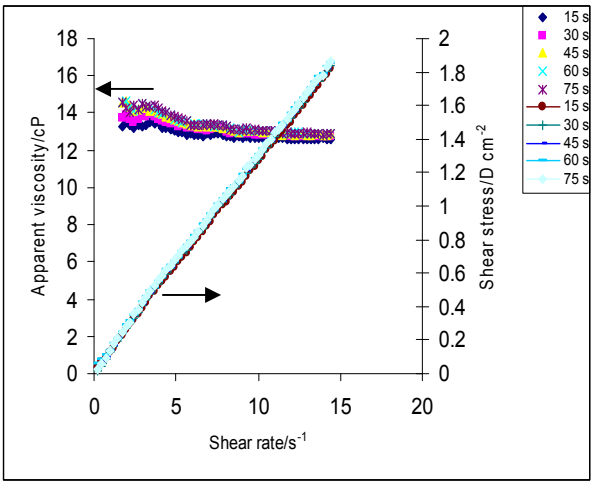
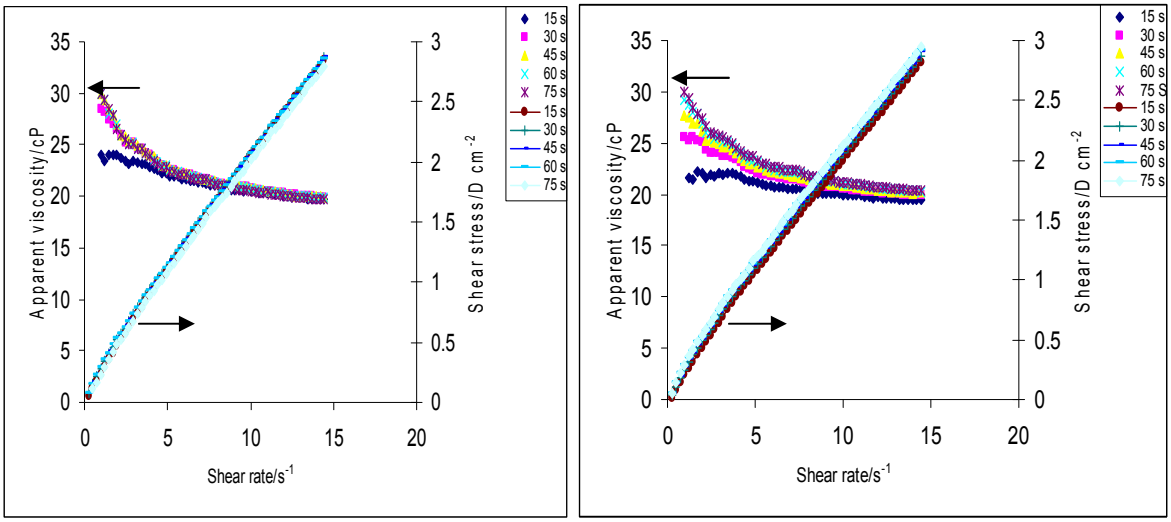


(d)



(e) (f)

Figure 4.14: The apparent viscosity and shear stress versus shear rate at various shearing times of: (a) pure CS and its blended solutions with AG and PVA at various proportions: (b) (90/05/05), (c) 80/10/10, (d) 70/15/15, (e) 60/20/20 and (f) 50/25/25 at 40^{o}C.

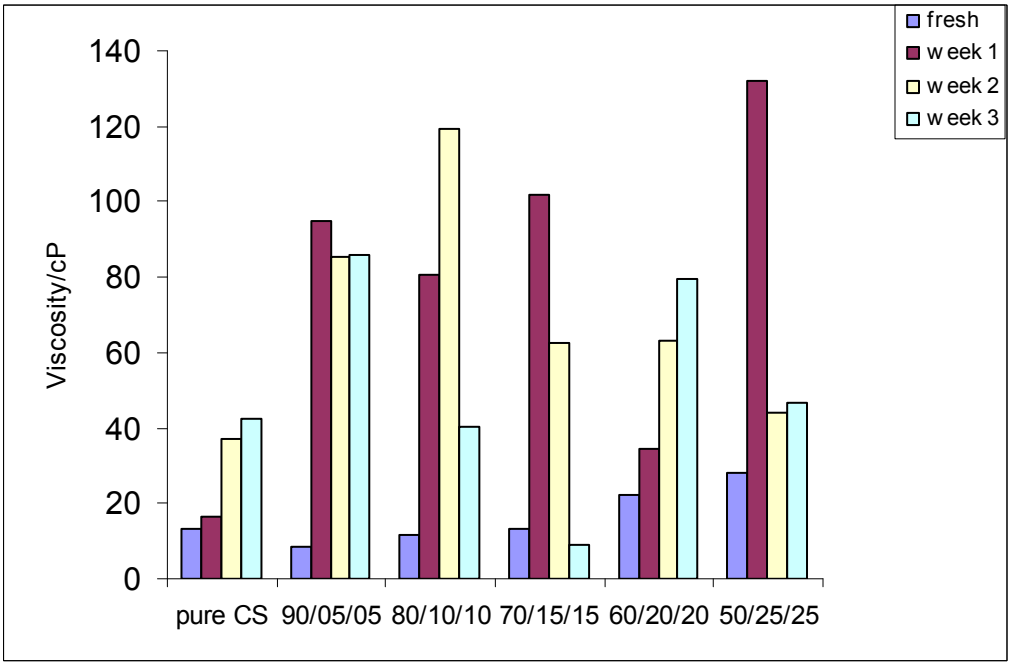


Figure 4.15: The effect of storage time on the apparent viscosity (determined at a constant shear rate) of pure CS and CS/AG/PVA ternary blended solutions.

5.0 Chapter 5: Characterizations of chitosan/agar (CS/AG) blended films

5.1 FTIR Analysis

5.1.1 FTIR Analysis of the pure components

Figure 5.1 shows a typical spectrum of pure CS film. The broad band at 3367 cm^{-1} is due to the OH stretching. The band at 1561 cm^{-1} is assigned for the NH bending (amide II) (NH_2) while the small peak at 1647 cm^{-1} is attributed to the C=O stretching (amide I) O=C-NHR. The bands at 2927, 2884, 1411, 1321 and 1260 cm^{-1} are assigned to CH_2 bending due to pyranose ring (Pawlak and Mucha, 2003). The band at 1380 cm^{-1} is due to CH_3 wagging. The characteristic features of chitosan spectrum in this study are similar to previous reports (Nunthanid *et al*, 2001; Ritthidej *et al*, 2002; Xu *et al*, 2005).

The FTIR spectrum of AG film is shown in Figure 5.2. The absorption band at about 3400 cm^{-1} is associated with O-H stretching (Tako *et al*, 1999) while the peak at 2900 cm^{-1} is attributed to methoxyl groups (Armisen and Galatas, 1987). The band at around 1643 cm^{-1} is due to the stretching of the conjugated peptide bond formed by amine (NH) and acetone (CO) groups (Cristiaen and Bodard, 1983). The peak at 1373 cm^{-1} is assigned to ester sulfate (Armisen and Galatas, 1987) and the bands at 1070 and 930 cm^{-1} are associated with the 3,6-anhydro-galactose bridges (Chirapart *et al*, 1995).

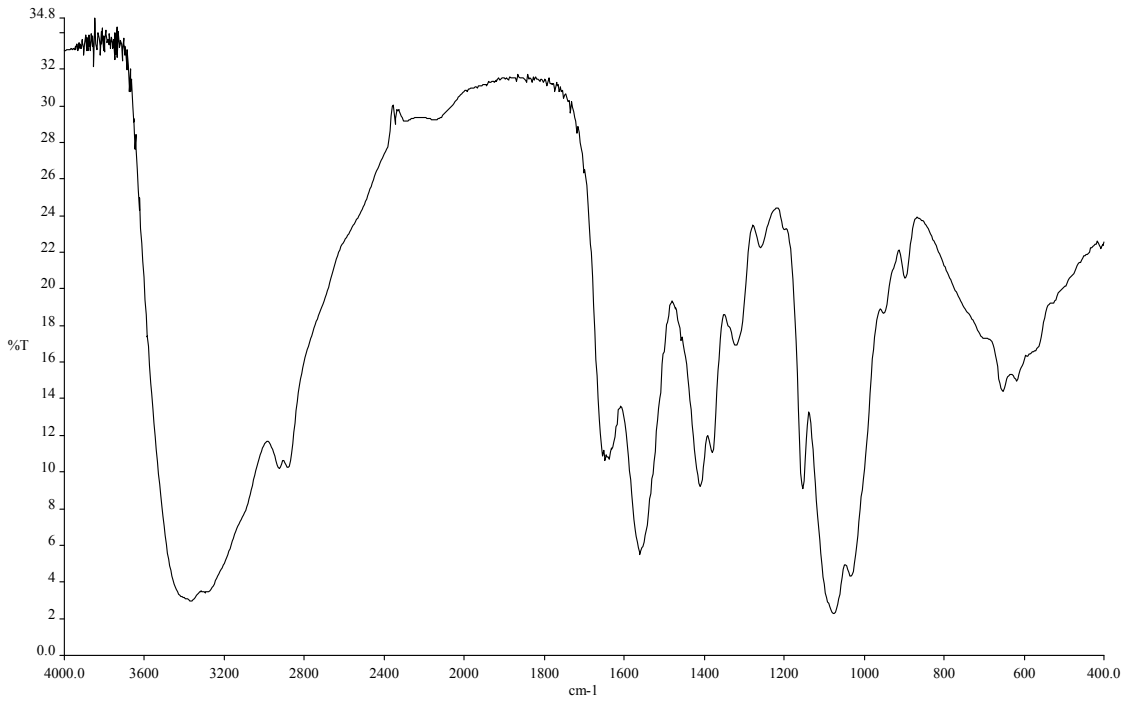


Figure 5.1: Typical FTIR spectrum of pure CS film.

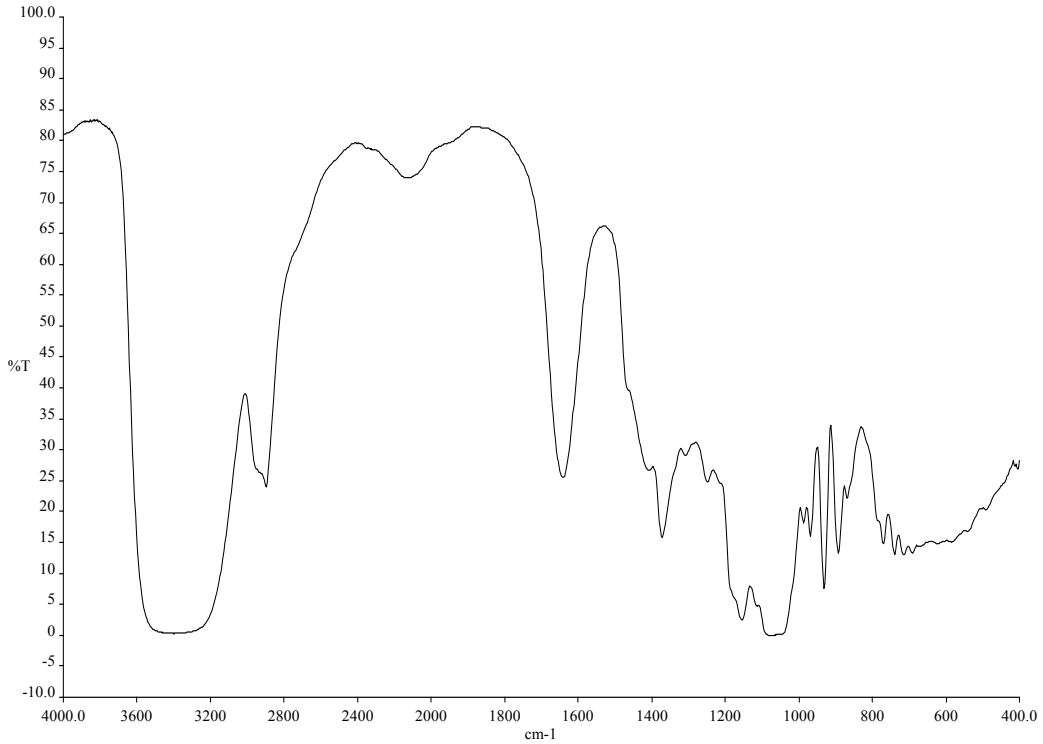


Figure 5.2: Typical FTIR spectrum of pure AG film.

5.1.2 FTIR Analysis of CS/AG blended films

Figure 5.3 shows the FTIR spectra of CS and AG blended films containing various AG proportions and their corresponding pure films. Typical features of CS and AG according to the summary of characteristic bands presented in Table 5.1 can be observed. The increase in the AG concentration in the blended films caused a decrease in the intensity of the band arising from the NH bending (amide II) at 1561 cm^{-1} of CS. This was coupled with a similar decrease in the absorbance band at 1411cm^{-1} and an increase in the band absorbance at 1380 cm^{-1}. Furthermore, the spectra of blended films were marked by the disappearance of the intensive band at 1033 cm^{-1} that was obviously observed the pure CS film and CS/AG blends containing AG concentration of 10%. This is due to the fact that mixing of two or more polymers brings about changes in characteristic spectra peaks caused by the reflection of the physical blends and chemical interactions (Guan *et al*, 1998; Yin *et al*, 1999). These observations indicate the existence of good miscibility between chitosan and agar and this is most likely due to the formation of hydrogen bonds between the amino and hydroxyl groups in CS and the hydroxyl groups in AG.

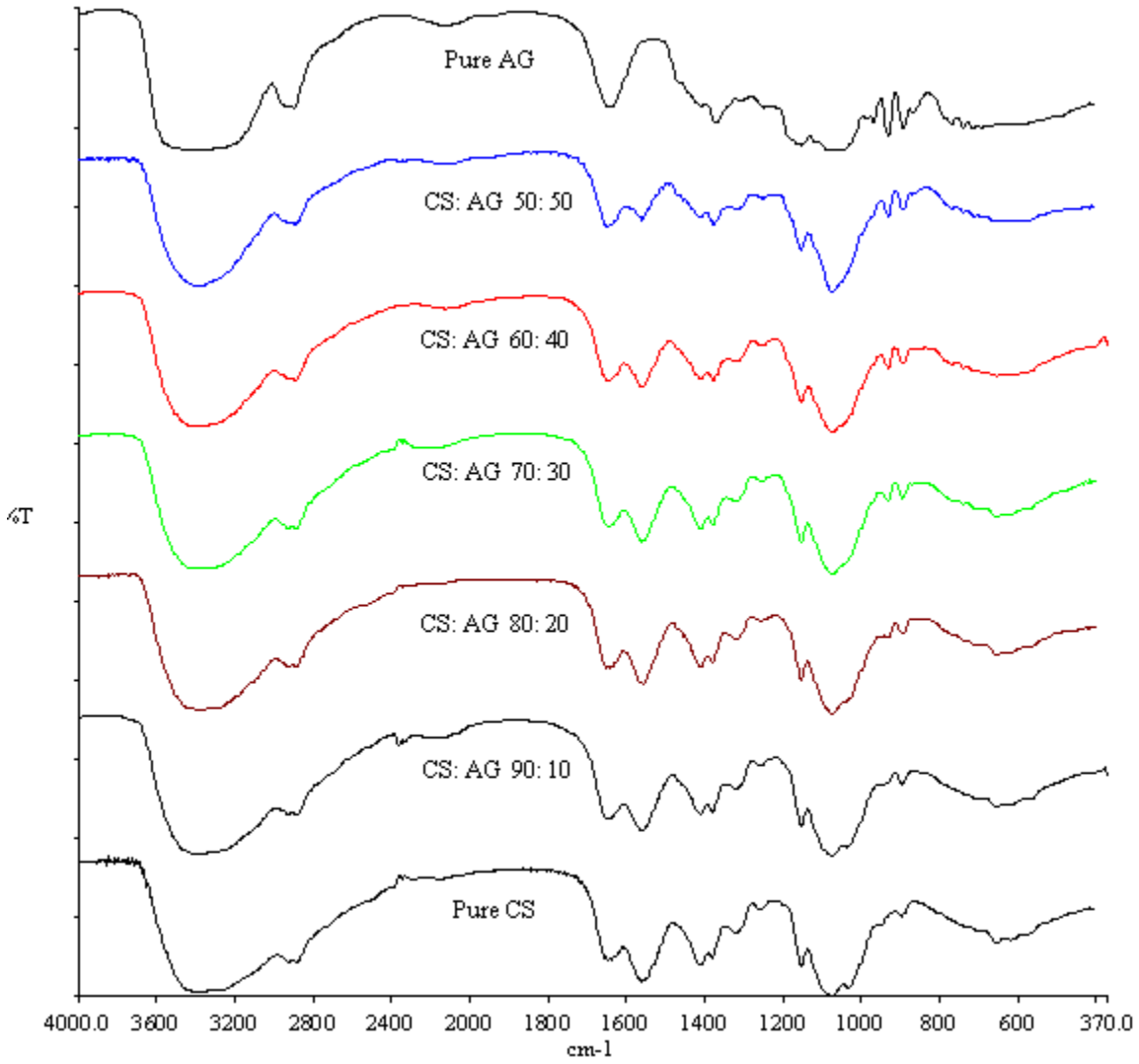


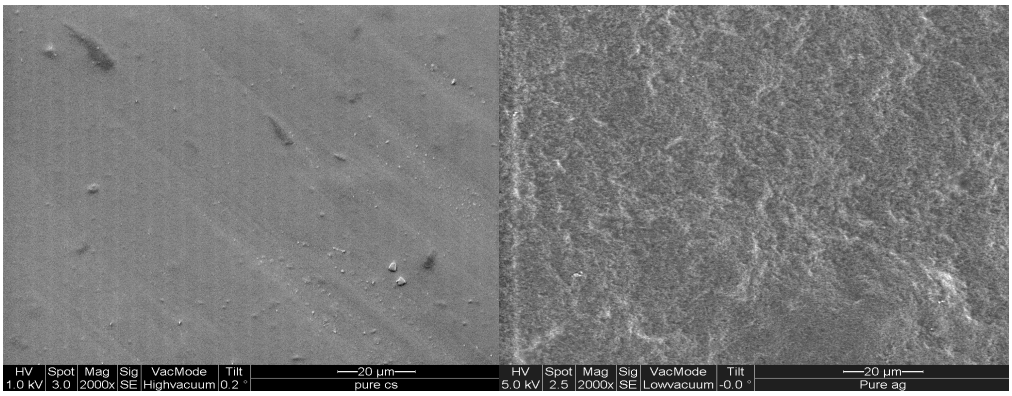
Figure 5.3: Typical FTIR spectra of pure chitosan and pure agar together with their blended films having various proportions

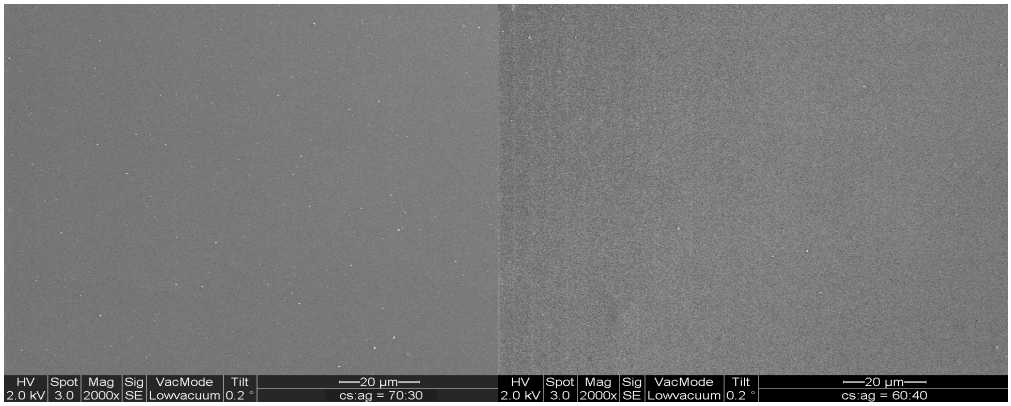
Table 5.1: FTIR characteristic bands (cm^{-1}) of chitosan/agar blended films with different proportions and their pure components.

CS/AG (%)	OH	C=O (amide I)	NH (amide II)	CH (amide II)	C-C	C-O
100/0	3368	1647	1561	1411	1380	1033
90/10	3368	1647	1559	1412	1379	1035
80/20	3371	1651	1558	1411	1379	-
70/30	3368	1646	1559	1411	1379	-
60/40	3371	1647	1560	1412	1378	-
50/50	3379	1647	1559	1417	1376	-
0/100	3400	1643	-	1409	1373	-

5.2 SEM measurements

Figure 5.4 shows the SEM micrographs of surfaces of CS/AG blended films containing different proportions with the corresponding pure components. As can be seen, chitosan film shows smooth and homogeneous surface with some parallel straps unlike that of pure agar which exhibits rougher surface. The surfaces of the blended CS and AG films seem to have no interface layer and more homogeneous than that of pure CS and AG ones. This confirms that CS and AG are highly compatible. The formation of homogeneous blends of CS and AG was mostly caused by the formation of hydrogen bonds between the functional groups of the blend component (–OH and $–NH_2$ groups in CS and –OH groups in AG).





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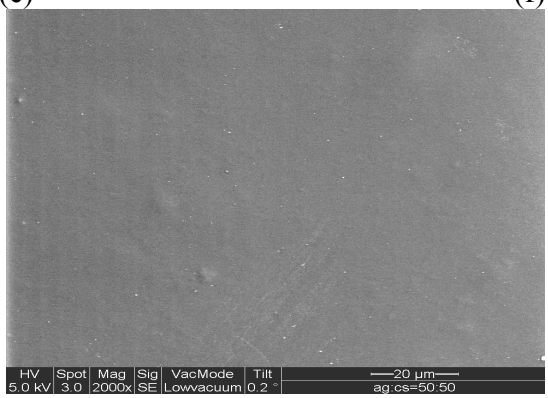


(g)

Figure 5.4: The scanning electron micrographs of: (a) pure CS and (b) pure AG films together with their blended films with various proportions: (c) 90/10, (d) 80/20, (e) 70/30, (f) 60/40 and (g) 50/50.

5.3 Tensile strength and elongation % at break

The variation of tensile strength and the elongation % at break of the CS/AG blended films with different agar proportions are shown in Figures 5.5 and 5.6. It was found that pure CS film recorded the highest value of tensile strength, *i.e.*, 25.0 MPa as expected. As the amount of AG increased, the tensile strength decreased substantially until agar content reached 30% in the blended film wherein it remains almost constant. The elongation % dropped from 52.5% for pure CS film to 24.3% for 80/20 CS/AG blended film after which it remains nearly constant despite increasing the AG content. This indicates that the blended films were found to be more brittle than pure CS film. However, the reduction in both

tensile strength and elongation% at break in the blended films is considered to be acceptable taking into consideration the nature of the two blended polysaccharide polymers. The reduction in both mechanical properties investigated in this work is most likely caused by the disruption in the crystallinity of the CS accompanied the intermolecular interactions of AG with chitosan with increasing AG content. The absence of significant changes in the mechanical properties when more than 30% of AG was blended with CS suggests the presence of possible crystalline structure dilution without considerable disruption in CS crystallinity. Similar behavior was reported in the literature for CS associated with other polymers such as polyethylene oxide (PEO). For example, Zivanovic *et al.* (2007) reported a general decrease in tensile strength and elongation % for CS/PEO blends as the PEO concentration increased. Costa-Junior *et al.* (2009) reported that CS/PVA blends exhibited intermediate mechanical properties between the pure components.

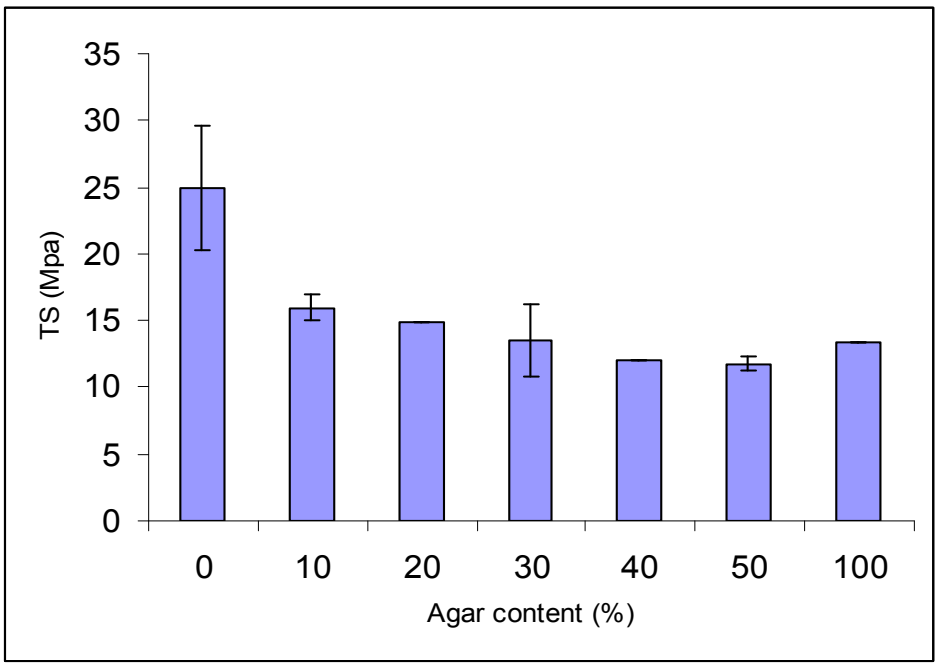


Figure 5.5: Tensile strength of chitosan/agar blended films versus agar content (0% = pure CS and 100% = pure AG) (pure agar and chitosan films are included as references).

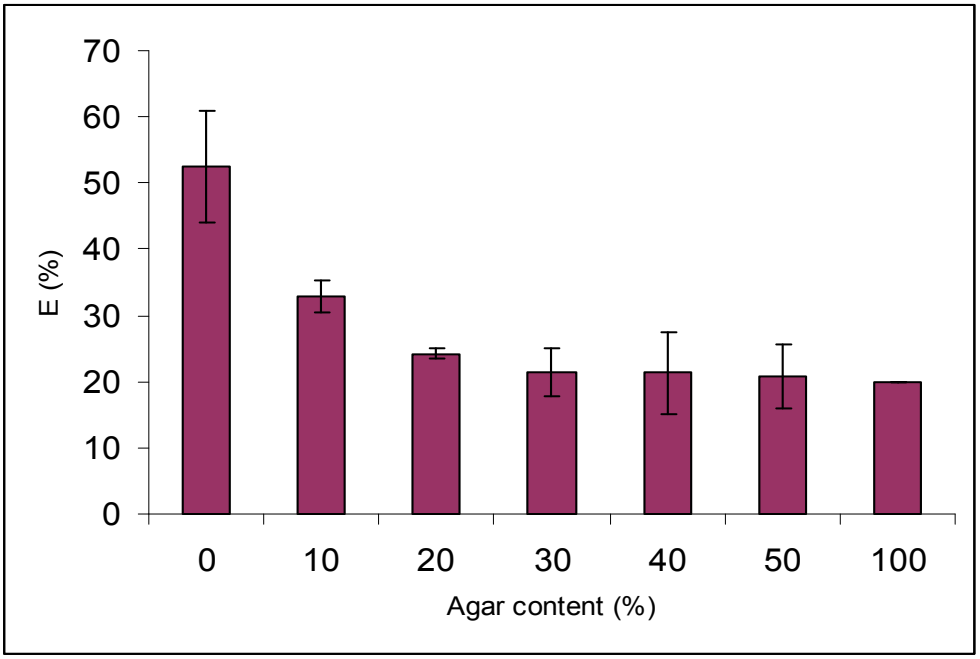


Figure 5.6: Elongation % of chitosan/agar blended films versus agar content (0% = pure CS and 100% = pure AG) .

5.4 TGA measurements

5.4.1 TGA of pure components

Figure 5.7 shows the results of thermogravimetric analysis of pure chitosan film. Two weight loss transitions are observed in the TGA curve of pure CS. The first transition is in the range of 40 - 130ºC and shows about 6% loss in weight. This is thought to be due to the moisture vaporization. The second weight loss begins at about 150^{o}C and the corresponding weight loss of about 52%, is attributed to the decomposition of chitosan polymer chanes. The total weight loss of the sample at about 500^{o}C is 58%. The remaining residue of the film which equals 42% is mostly due to the formation of inorganic complex containing C, N and O. Similar behavior of chitosan film was reported in the literature (Liu *et al*, 2002; Britto and Campana-Filho, 2004; Chen *et al*, 2008b).

The TGA curve of AG film showed two distinct zones within the range of investigated temperatures (Figure 5.8). The initial slight loss in the weight (about 5% and continue up to 130^{o}C) is attributed to the loss of water. This followed by a rather sharp break at around 210^{o}C indicates the onset of a decomposition process involving a rapid loss in weight (around 61% at 500^{o}C). These results are also in agreement with previous studies reported in literature (Athawale *et al*, 1999; Kurose *et al*, 2004).

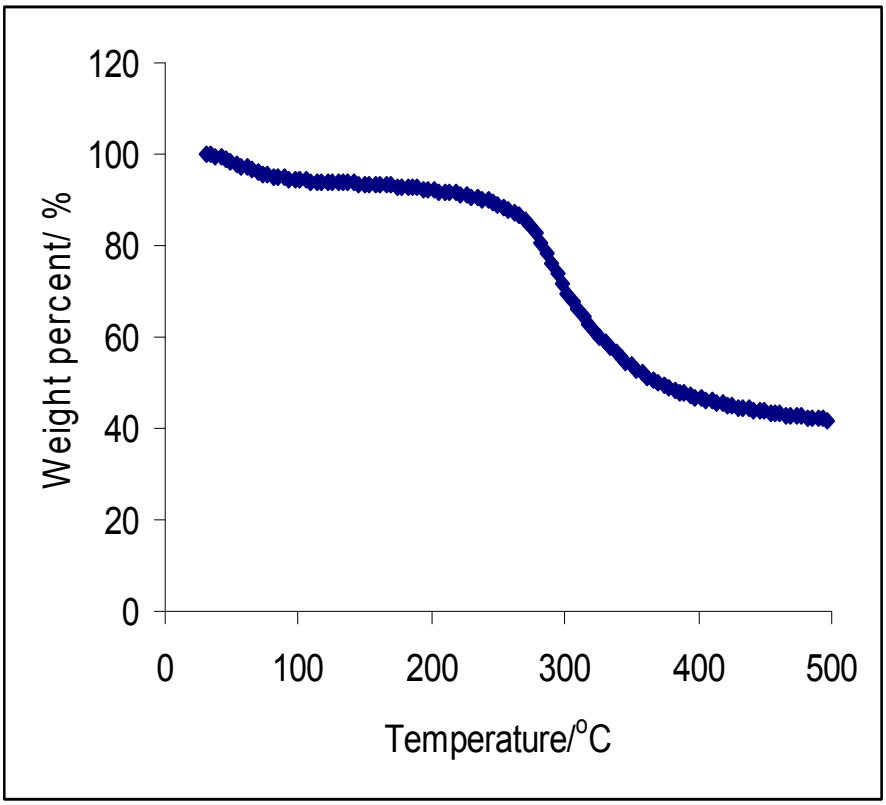


Figure 5.7: TGA curve of pure chitosan film.

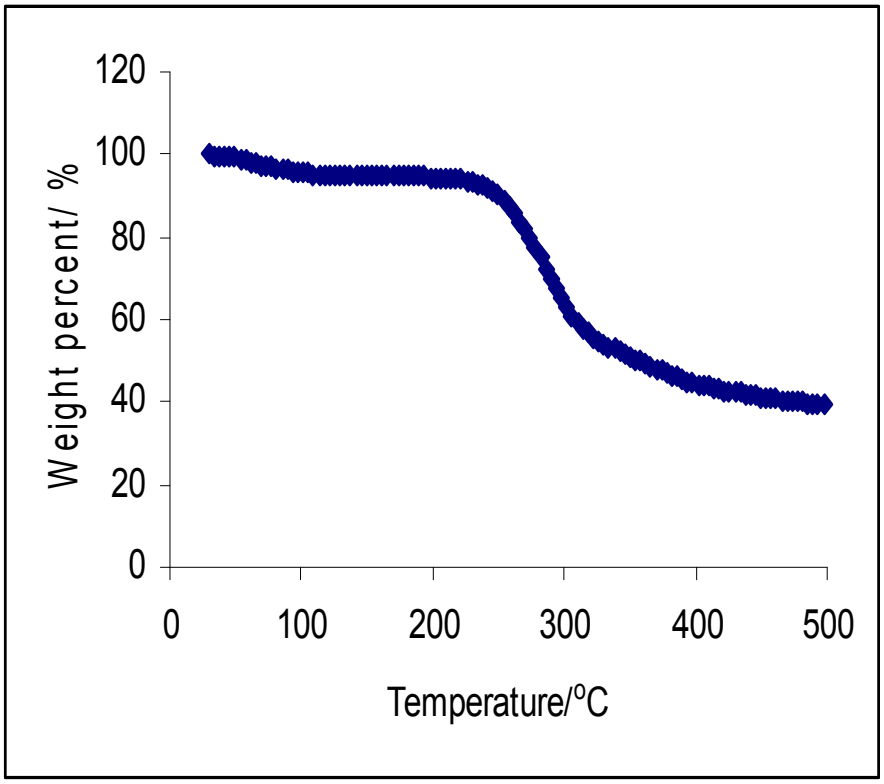


Figure 5.8: TGA curve of pure agar film.

5.4.2 TGA of CS/AG blended films

Figure 5.9 shows the results of thermogravimetric analysis of CS/AG blended films and their pure components and the data of the TGA of all films are summarized in Table 5.2. From the table, it is clear that the blended film with ratio 80/20 showed the lowest amount of water content unlike films containing higher ratios, which showed higher water content compared to their pure components. This trend indicates that an increase in the hydrophilicity of the blended films took place, which might be caused by a slight deterioration in crystallinity of CS. It can also be noted that the ratio of 80/20 has the lowest weight loss almost at all temperatures studied indicating better thermostability. The continuous degradation observed in TGA curves of blended films together with the absence of any phase separation confirms that CS and AG form highly compatible blends. This is

highly associated with the interactions between AG and CS through hydrogen bonding formation between their functional groups (–OH and $–NH_2$ groups in CS and –OH groups in AG) (Wu *et al*, 2008).

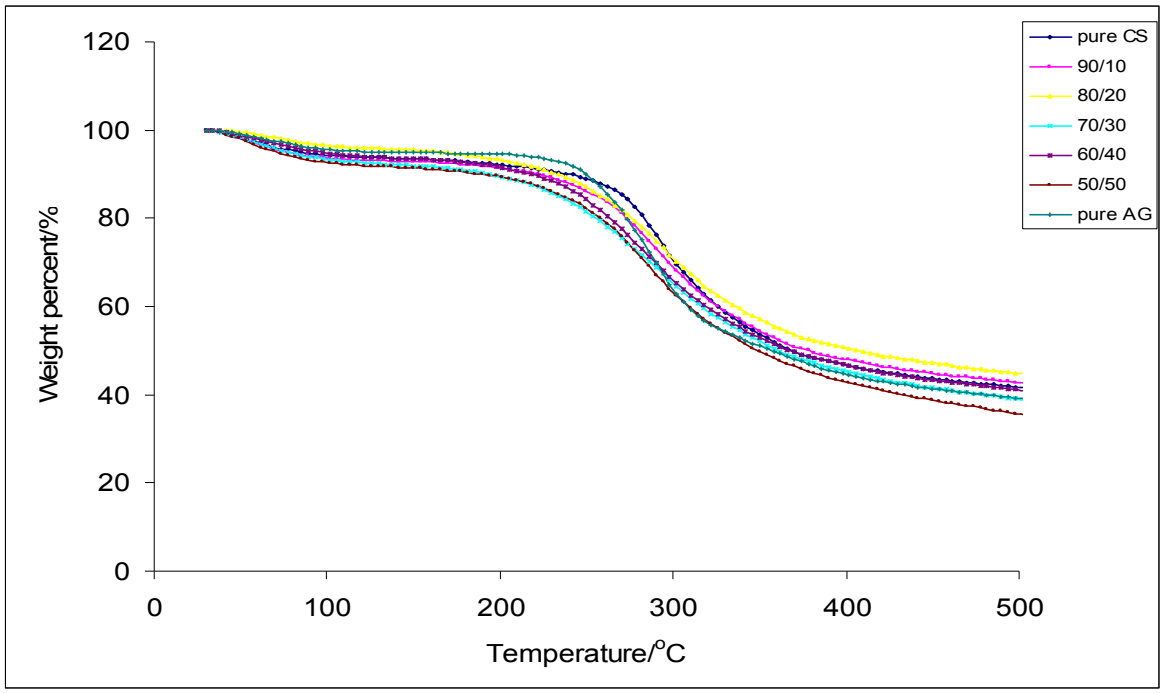


Figure 5.9: TGA curves of chitosan/agar blended films and their pure components.

Table 5.2: The weight loss (%) of the pure chitosan and agar films together with their blended ones having various ratios at different temperatures.

Temperature (°C)	Pure CS	90/10	80/20	70/30	60/40	50/50	Pure AG
100	5.7	6.5	3.6	6.7	5.3	7.4	4.4
200	7.9	8.7	6.9	10.6	8.5	10.7	5.5
300	30.4	31.8	29.7	35.5	34.8	37.6	37.1
400	53.5	52.2	49.6	54.8	53.5	57.4	55.6
500	58.3	57.2	55.1	61.0	59.0	64.4	60.8

5.5 DSC measurements

Figure 5.10 shows the DSC curves obtained from the first run of the pure CS and pure AG films together with their blended films having various ratios. The DSC curve of CS film shows a broad endothermic peak at around 79^{o}C while AG film shows a smaller endothermic peak at about 75^{o}C. All compositions exhibited a broad endothermic peak at different positions ranging from about 75 to 84^{o}C (Table 5.3). Some endothermic peaks were reported in the literature for CS film. For example, Lima *et al.* (2006) and Wang *et al.* (2003) reported an endothermic peak of CS film at 60.75 and 83^{o}C for fully deacetylated CS and 85% deacetylated one respectively. As for pure AG, Eldridge and Ferry (1954) demonstrated that AG film exhibits an endothermic peak at around 90^{o}C while Lyons *et al.* (2009) found that the AG film shows an endothermic peak at 110^{o}C, which is higher than the usual reported value. Also, Suzuki *et al.* (2001) reported endothermic peaks of three kinds of AG gel at different temperatures ranging from 75 to 90°C. According to some reports, this endothermic peak is related to the melting temperature (Nugraha *et al*, 2004). However, it is often termed as dehydration temperature (T_D), which is attributed to the evaporation of water associated with the hydrophilic groups of the polymers (Gonzalez *et al*, 2000, Cheung *et al*, 2002) and reflects the strength of water–polymer interaction (Sakurai *et al*, 2000, Kittur *et al*, 2002). This suggests that some bound water was still not removed from the samples when dried in the desiccator. These endothermic peaks were absent in the second run curve (Figure 5.11) which confirmed that this peak is attributed to the water associated with the polymer function through hydrogen bonding. A closer look of Figure 5.10 reveals that there is a difference in the endothermic peak area for the films, *i.e*., they vary in their water-holding capacity, showing that the blended films have higher water content in general than the pure components. This could be due to the formation of new hydrophilic centers (Neto *et al*, 2005). Another point to be noted is that all films with

various ratios have values of T_D close to that of the pure polymers except for the ratio 60/40, which shows a higher value. The shift in the peak position to a higher temperature indicates a stronger interaction with water. It is believed that this variation on the position and peak area is due to the physical and molecular changes caused by blending of the two poymers. These results suggest that the presence of an interaction between CS and AG may occur to form films with better thermostability.

With respect to the glass transition temperature (T_g) of CS, various values were reported in literature ranging from -23 to 220^{o}C (Ogura *et al*, 1980, Pizzoli *et al*, 1991; Ratto *et al*, 1995; Sakurai *et al*, 2000; Nugraha *et al*, 2004; Lazaridou and Biliaderis, 2005) as shown in Table 5.4. The variations in the values of T_g of CS is probably due to the difficulty in identifying it due to the presence of large amount of water (Chen *et al*, 2008b) that are involved in intra-and intermolecular hydrogen bonds in polymer chain (Cai and Kim, 2008). In addition, being a natural polysaccharide, the source and the method of preparation of CS can influence its T_g (Neto *et al*, 2005). In this work, the T_g of CS and its blends could not be detected.

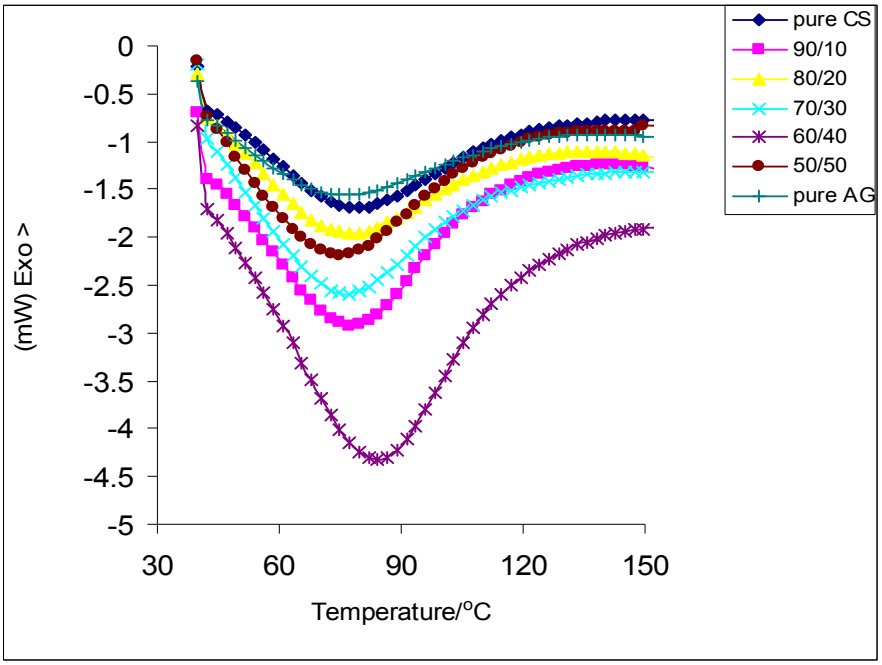


Figure 5.10: The DSC first - run curves of the pure chitosan and pure agar films together with their blended films.

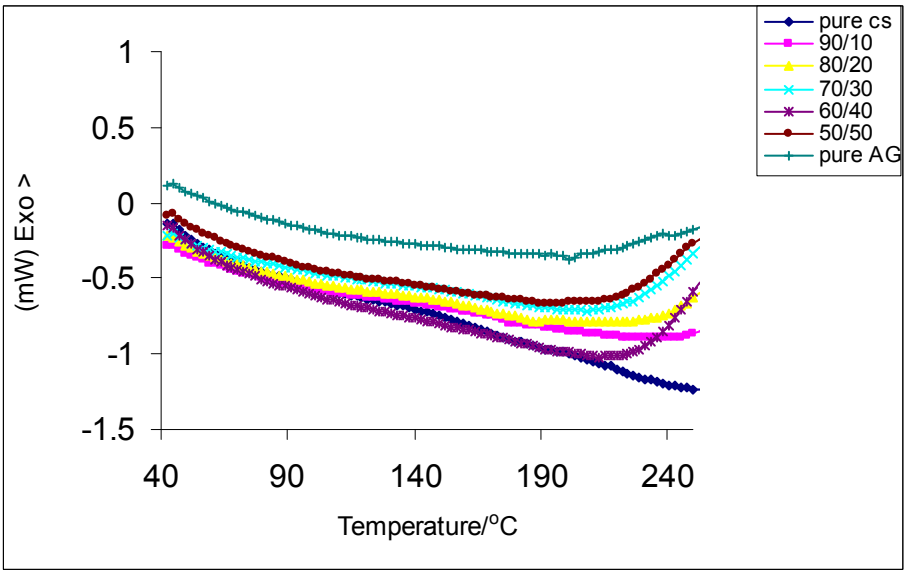


Figure 5.11: The DSC second - run curves of the pure chitosan and pure agar films together with their blended films.

Table 5.3: The thermal properties of the pure chitosan and pure agar films together with their blended films with different agar contents.

Blend composition CS/AG	100/0	90/10	80/20	70/30	60/40	50/50	0/100
T_D (^oC)	79.0	78.4	77.4	75.7	83.8	74.8	75.3
ΔH (J/g)	227.6	235.9	260.6	336.6	362.5	325.3	240.5

Table 5.4: The values of T_g of the pure chitosan reported in the literature.

T_g (^oC)	194	150	~220	30	from -23 to 67	203
Ref	(Cheung *et al*, 2002)	(Gonzalez *et al*, 2000)	(Kittur *et al*, 2002)	(Sakurai *et al*, 2000)	(Neto *et al*, 2005)	(Suzuki *et al*, 2001)

5.6 Swelling behavior

Figure 5.12 shows the degree of swelling of pure and blended films versus the AG content. As can be seen, all blended films showed higher degrees of swelling compared to pure CS film, indicating higher hydrophilicity. The swelling degrees of the blended films range from 386 to 457 % while the swelling degrees of the pure CS and pure AG films are 89 and 620%, respectively. This is because AG is a water-soluble polymer and the blending of CS with AG tends to increase the water uptake due to the increasing of hydrophilic groups (-OH) in the blends and the associated high amorphous content. Similar behavior was reported for CS blended with the synthetic water-soluble polymer, PVA (Bahrami *et a*l, 2003; Wang and Gunasekaran, 2006) in which the water uptake was similarly found to increase with increase in PVA content.

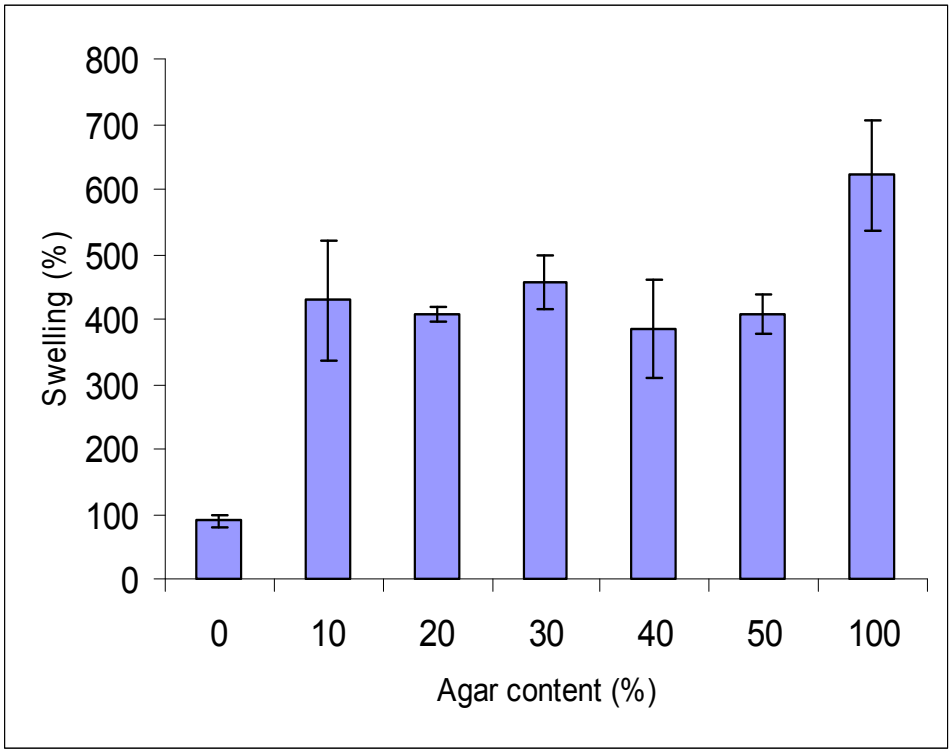


Figure 5.12: The degree of swelling of chitosan/agar blended films versus agar content (0% = pure CS and 100% = pure AG).

5.7 Contact angle measurements (surface hydrophilicity)

Figure 5.13 shows the relationship between the static water contact angle and the AG content for blended CS/AG films having various proportions. The plot shows that there is a general decrease in the static water contact angle with increasing the AG content in the blend *i.e.*, the surface becomes more hydrophilic when the concentration of AG increases in the blend. The contact angle of pure CS was $88.12^{o}\pm1.91$, which in agreement with the literature (Tangpasuthadol *et al*, 2003; De Britto and Assis, 2007) while the contact angle of the blended films ranged from 88.41± 2.90 to $72.03^{o}\pm$ 0.52. In general, the contact angles for all the blended films were less than 90^{o}, indicating good hydrophilicity of the surfaces.

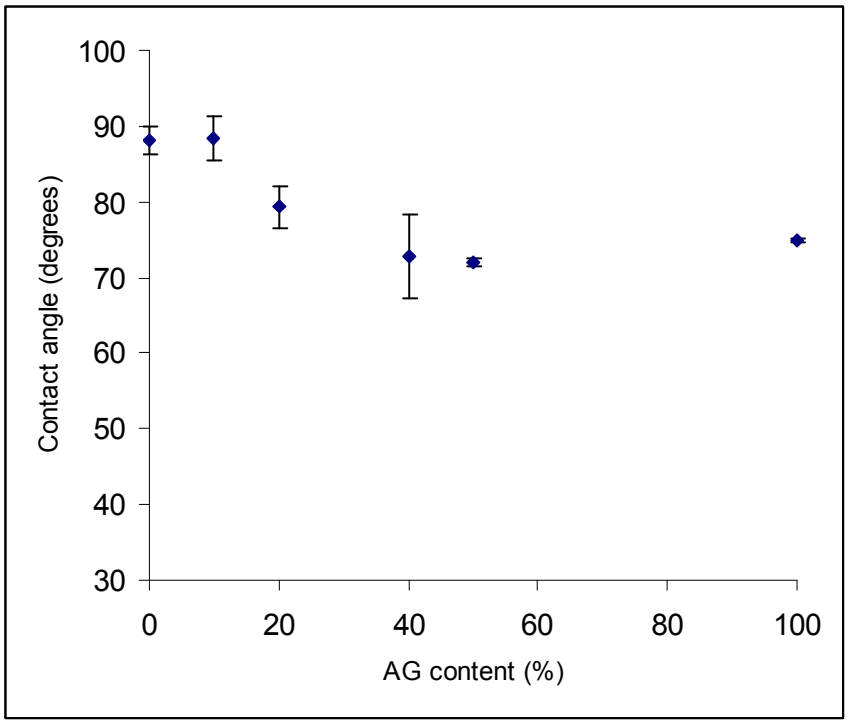


Figure 5.13: The relationship between the water-contact angle and the agar content for chitosan/agar blended films having various proportions (0% = pure CS and 100% = pure AG).

6.0 Chapter 6: Characterizations of chitosan/poly (vinyl alcohol) (CS/PVA) blended films

6.1 FTIR Analysis

6.1.1 FTIR Analysis of the pure PVA film

Figure 6.1 shows a typical FTIR spectrum of pure PVA film. As can be seen, there is an absorption peak at around 3368 cm^{-1}, which refers to the intermolecular hydrogen bonding and –OH stretch vibration (Ahmad and Ooi, 2005). The vibrational band observed at 2941 cm^{-1} is associated with the C-H stretching from alkyl groups. The absorption corresponding to the -C-O stretching occurs at 1096 cm^{-1}.

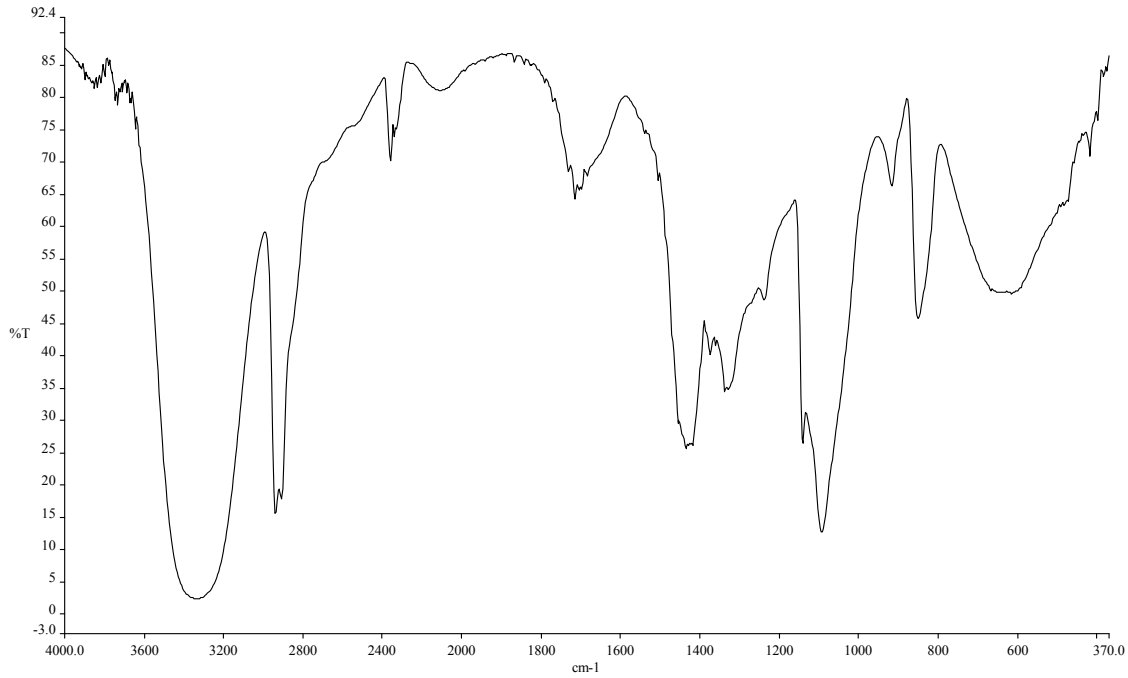


Figure 6.1: Typical FTIR spectrum of pure PVA film.

6.1.2 FTIR Analysis of CS/PVA blended films

Figure 6.2 shows the FTIR spectra of pure CS and pure PVA together with their blended films containing various PVA proportions. A summary of characteristic bands of chitosan and PVA blended films is also presented in table 6.1. As can be seen, the increase in the PVA concentration in the blend films caused a decrease in the intensity of the band arising from the NH bending (amide II) at 1561 cm^{-1} of chitosan. Also, an increase in the intensity of CH group at around 2928 cm^{-1} was observed as the PVA content increases. In addition, there was a remarkable shift for the peak at 1077 cm^{-1} to a higher wave number with the increase of PVA content in the blend. Furthermore, the band at 850 cm^{-1} disappeared in the spectra of the pure chitosan film and the chitosan/PVA blend film containing PVA concentration of 10%. However, the intensity of this band was increasing for the other ratios as the content of PVA increased. This is attributed to the changes in characteristic spectra peaks, which occur after blending due to the reflection of the physical blends and chemical interactions (Guan *et al*, 1998; Yin *et al*, 1999). These observations indicate the existence of good miscibility between chitosan and PVA due to the formation of intermolecular hydrogen bonds between the amino and hydroxyl groups in chitosan and the hydroxyl groups in PVA. Similar observations were obtained for the CS/AG blend films mentioned in the previous chapter.

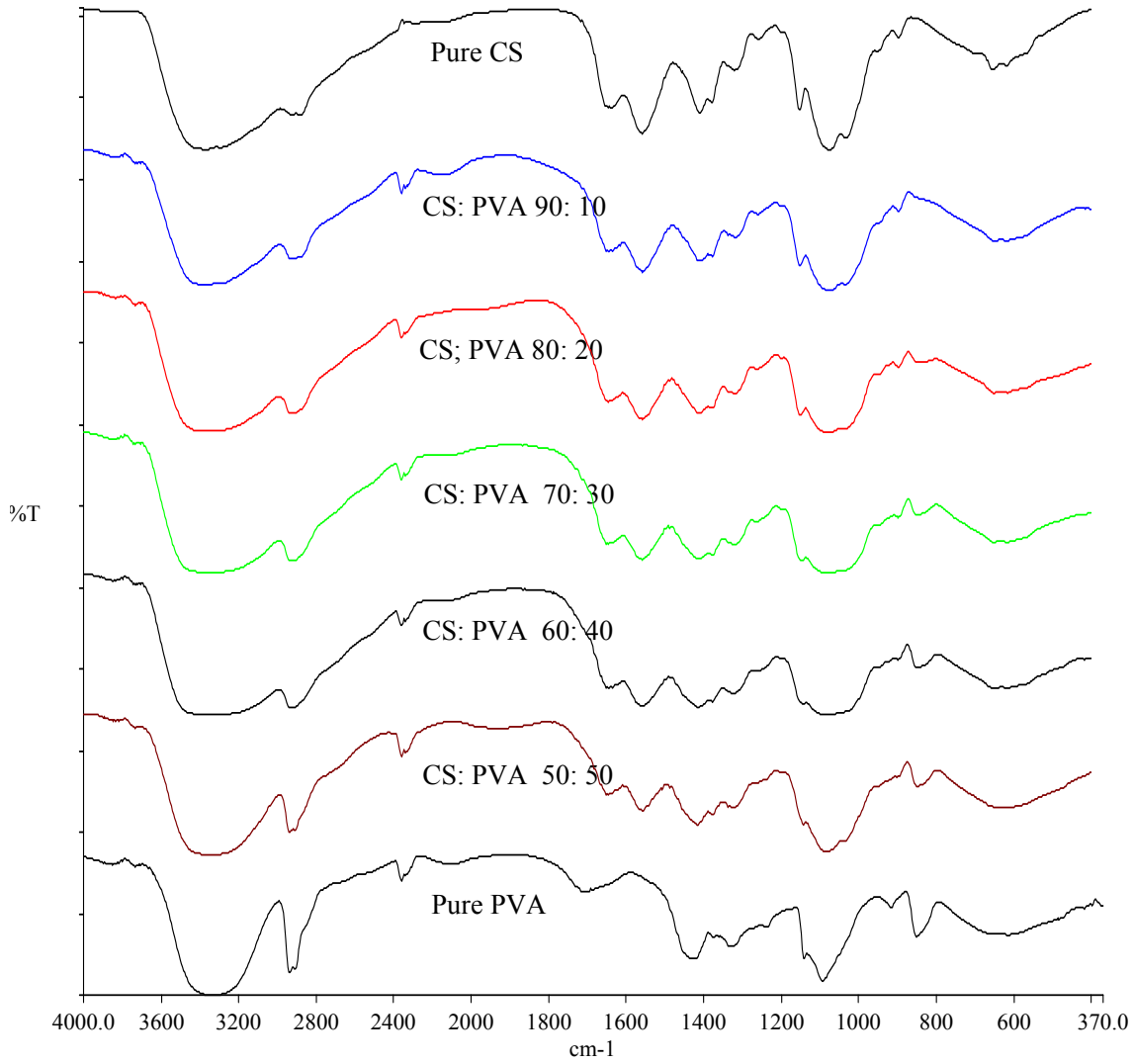


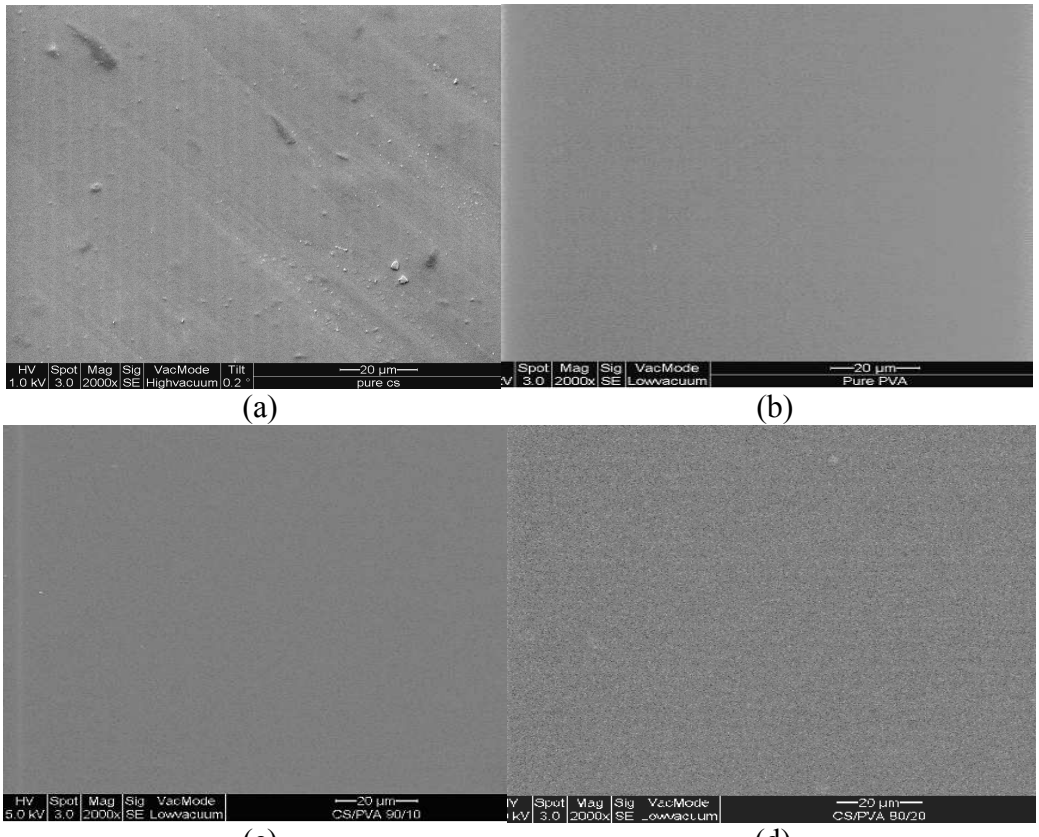
Figure 6.2: Typical FTIR spectra of pure chitosan and pure PVA together with their blended films having various proportions.

Table 6.1: FTIR characteristic bands (cm^{-1}) of chitosan/PVA blended films with different proportions and their pure components.

CS/PVA (%)	C-C	C-O	C-O stretch	CH	NH amide II	C=O (amide I)		CH	OH
100/0	-	1033	1077	1380	1561	1647	-	2928	3368
90/10	-	1035	1077	1386	1559	1647	2360	2927	3367
80/20	850	1035	1078	1379	1559	1647	2360	2935	3367
70/30	851	1036	1079	1379	1559	1647	2360	2937	3367
60/40	850	1039	1082	1380	1559	1647	2361	2939	3367
50/50	851	1037	1087	1379	1559	1647	2360	2940	3367
0/100	852	-	1096	1375	-	-	2360	2941	3368

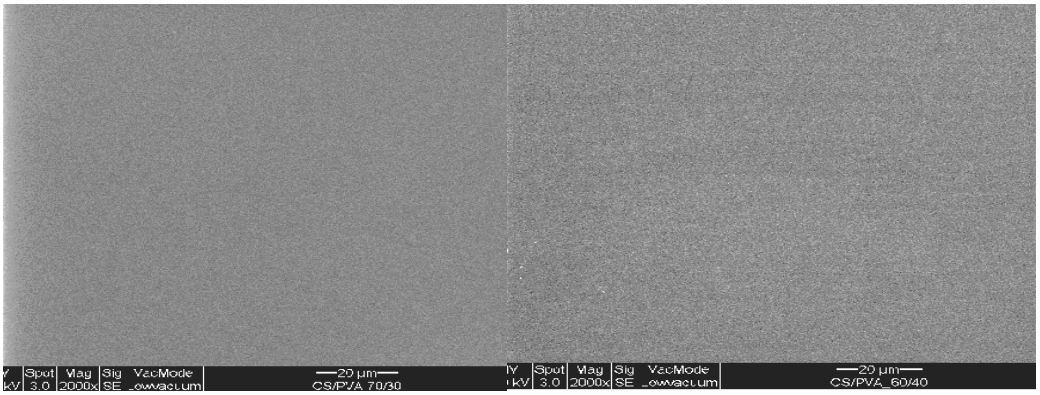
6.2 SEM measurements

The SEM images of the top surfaces of pure CS, pure PVA and CS/PVA blended films at different proportions are shown in Figure 6.3. As can be seen, CS film shows smooth and homogeneous surface with some straps on the top surface unlike that of pure PVA where no straps is seen. The surfaces of the blends of CS and PVA are homogeneous with no pores (Peng *et al*, 2007) and have no interface layer. However, the blended films also exhibit little flat smooth surfaces in general indicating the uniform distribution of CS and PVA molecules throughout the films. The formation of homogeneous blends of CS and PVA was mostly caused by the interactions of hydrogen bonds between the functional groups of the blended components. Similar observations were obtained for CS/AG blended films in the previous chapter.



(a) (b)

(c) (d)



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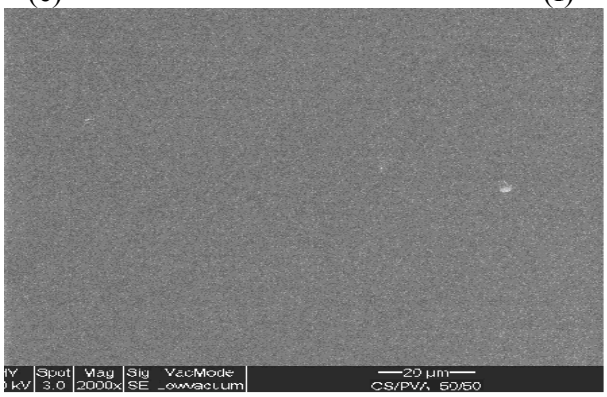

(g)

Figure 6.3: The scanning electron micrographs of: (a) pure CS film and (b) pure PVA film together with their blended films with various proportions: (c) 90/10, (d) 80/20, (e) 70/30, (f) 60/40 and (g) 50/50

6.3 Tensile strength and elongation % at break

The tensile strength and the elongation % at break of the CS/PVA blended films with different PVA proportions are shown in Figures 6.4 and 6.5. It was found that almost all the CS/PVA blended film recorded higher values than the pure components. The blend 90/10 recorded the highest value of tensile strength, *i.e*., 58.7 MPa, after, which the tensile strength decreased gradually as the amount of PVA increased. This shows that blending improves strength that increases with increasing PVA content up to 40%. This improvement in tensile strength is due to the interaction between –OH and $–NH_2$ groups of CS and –OH groups of PVA (Kim *et al*, 1992). It was also found that the pure PVA

recorded the highest percentage of elongation and that all the blended films recorded lower percentage of elongation than the pure components. This indicates that the blended films were more brittle and less flexible than the pure components. Similar behavior was reported in the literature for CS associated with PVA. For example, Park *et al.* (2001c) reported that PVA/CS blended film cast from acetic acid recorded higher values of TS and lower % elongation than the pure polymers. Hyder and Chen (2009) and Bahrami *et al*. (2003) also reported similar behavior of PVA/CS blended films with respect to tensile strength. However, different trend was observed for CS/AG blended films in the previous chapter in which there was a decrease in the TS and the elongation % with increasing AG content.

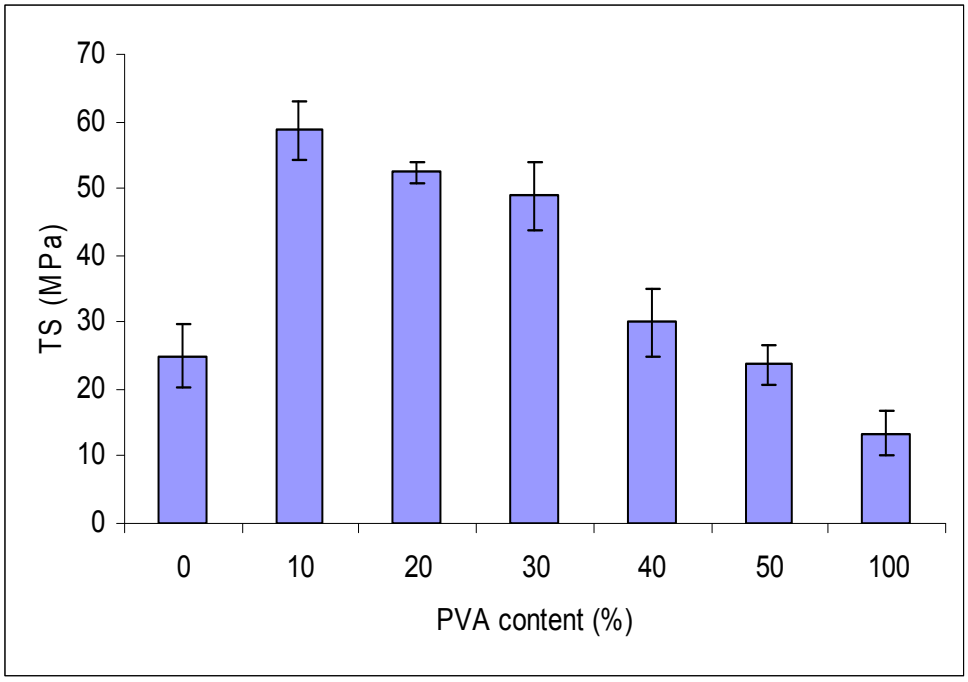

Figure 6.4: Tensile strength of chitosan/PVA blended films versus PVA content (0% = pure CS and 100% = pure PVA)

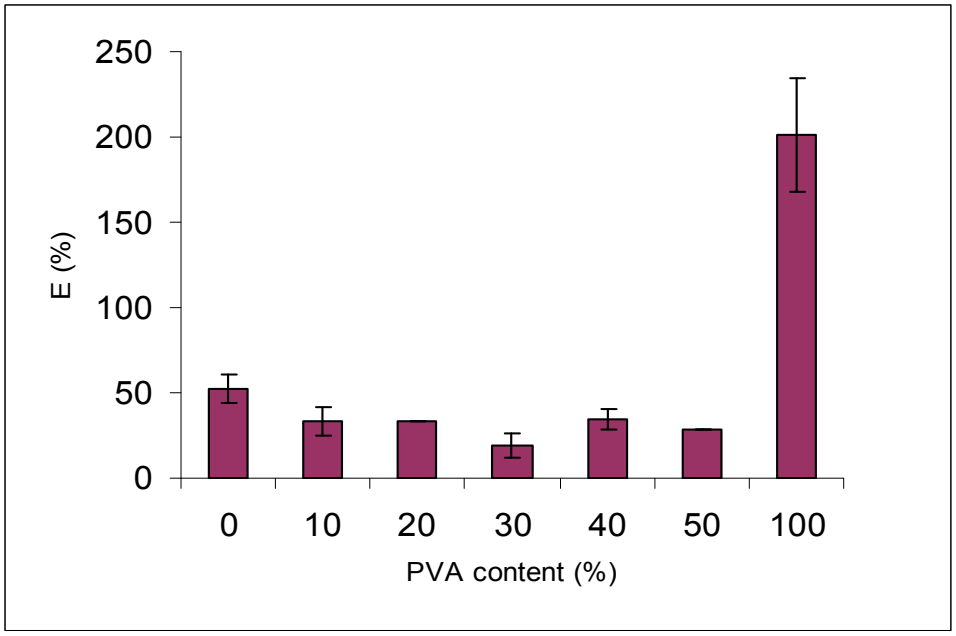

Figure 6.5: Elongation % of chitosan/PVA blended films versus PVA content (0% = pure CS and 100% = pure PVA)

6.4 TGA measurements

6.4.1 TGA of the pure PVA film

Figure 6.6 showes the TGA curve of the pure PVA film. As it can be seen, the pure PVA film exhibits a three-step degradation pattern. The first step began around 40^{o}C up to 79^{o}C with a weight loss up to 3%. The second and the major mass loss is between around 260^{o}C to 430^{o}C with a weight loss of about 73%. This was followed by a further smaller mass loss (around 17%) in the third step (from about 430^{o}C to 480^{o}C). The first step of weight loss could be attributed to the evaporation of loosely bound water. The second step is predominantly caused by the heat decomposition of the polymer structure. With further heating, the polymer backbone is broken down. It was reported that the third weight loss of PVA is related to the production of degradation products generated by PVA during the

TGA thermal degradation process (Holland and Hay, 2001). Similar observations have been reported in literature (Shi *et al*, 2008; Zhou *et al*, 2009).

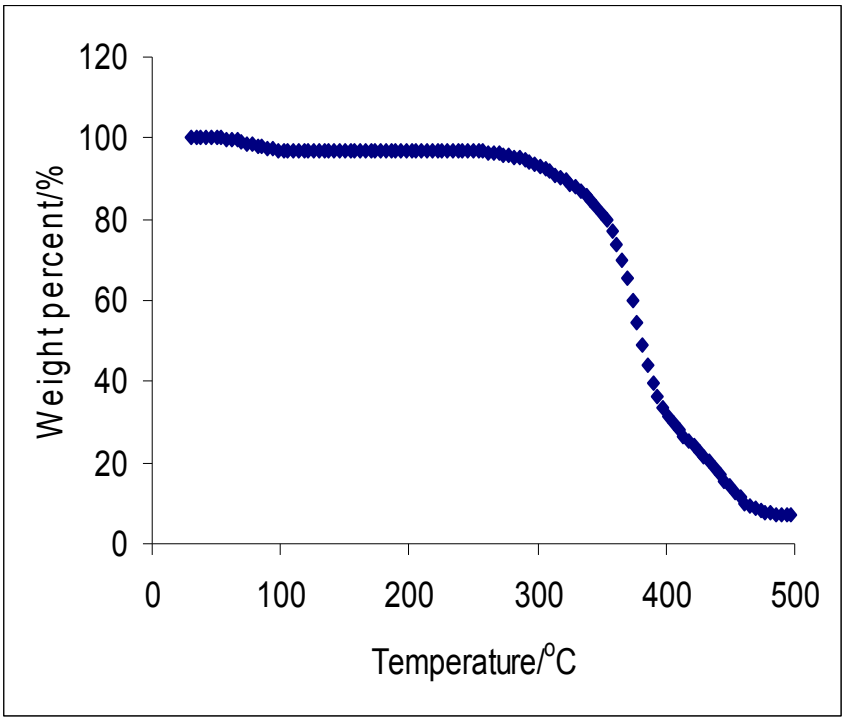

Figure 6.6: TGA curve of pure PVA film.

6.4.2 TGA of CS/PVA blended films

The representative TGA curves for the pure CS and pure PVA films together with their blended films at different ratios are shown in Figure 6.7. The weight loss data obtained from TGA for pure components and their blends are summarized in Table 6.2. It can be clearly seen that the blended film having CS/PVA ratio of 50/50 shows the lowest amount of water content unlike blended films containing other ratios, which show higher water content compared to the pure CS film. It can also be observed from figure 6.7 that all blends have only one onset indicating that interaction may take place between the two

components in each blend attributed to the formation of hydrogen bonding between the functional groups of the blended components. These observations show reasonable agreement with the CS/AG blended films.

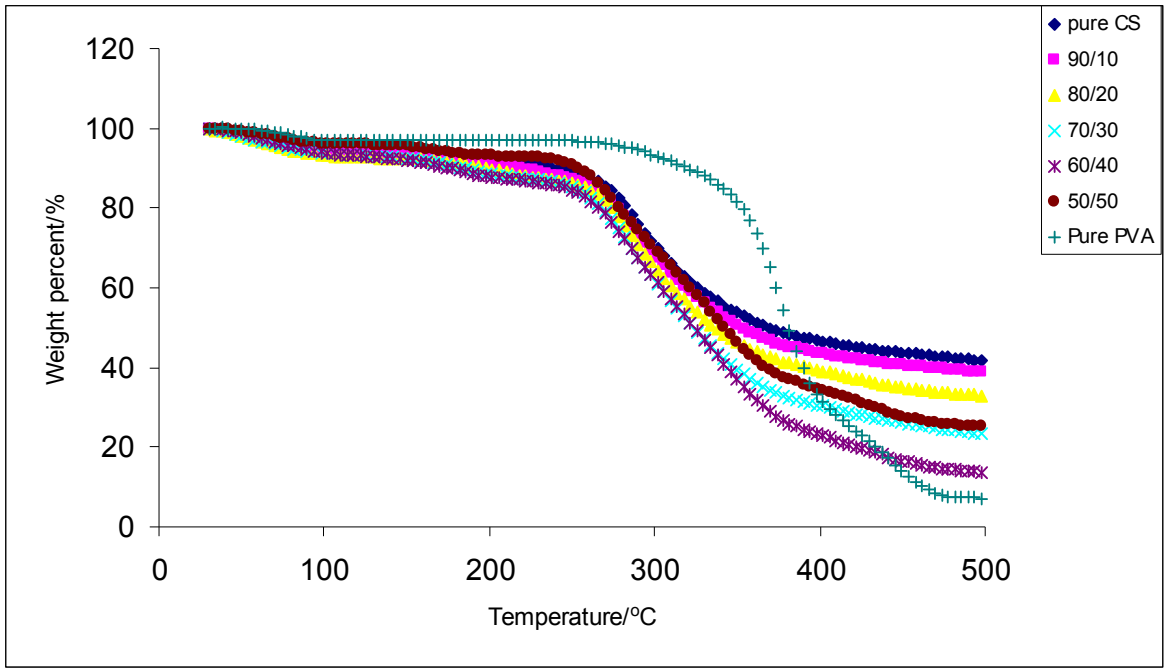

Figure 6.7: TGA curves of chitosan/PVA blended films and their pure components.

Table 6.2: The weight loss (%) of the pure chitosan and PVA films together with their blended ones having various ratios at different temperatures.

Temperature (°C)	Pure CS	90/10	80/20	70/30	60/40	50/50	Pure PVA
100	5.7	6.1	6.9	6.3	6.2	3.9	3.1
200	7.9	8.7	10.3	11.5	12.3	7.0	3.1
300	30.4	32.7	35.6	39.0	38.7	31.0	7.1
400	53.5	56.6	60.9	69.7	77.2	65.6	68.6
500	58.3	61.3	67.1	76.8	6.3	74.9	92.8

6.5 DSC measurements

Figure 6.8 shows the DSC curves obtained from the first run of the pure CS and pure PVA films together with their blended films having various ratios. The DSC curve of CS film shows a broad endothermic peak at around 79^{o}C while PVA film shows a smaller endothermic peak at 89^{o}C. All compositions exhibited a broad endothermic peak at lower position than the pure components ranging from about 70.3 to 77.3^{o}C (Table 6.3). Taking a closer look at figure 6.8 reveals that there is a variation in their water-holding capacity, showing that the pure CS film has the highest water content while the pure PVA has the lowest water content. Another point to be noted is that all ratios have lower values of T_D than the pure polymers. This variation on the position and peak area might be due to the physical and molecular changes caused by blending. These results suggest that the interaction between CS and PVA may occur to form more stable blend.

Since polysaccharides are apt to absorb moisture, a second heating run of the DSC, after heating to 150^{o}C, hold for a minute and then cooling to 40^{o}C, was done to eliminate the effect of moisture (Figure 6.9). The first point to note is the disappearance of the endothermic peak, confirmed that this peak is attributed to the water content in the sample. Also, pure PVA film exhibited a sharp endothermic melting transition at 222°C while pure chitosan film did not show any melting transition due to the fact that most polysaccharides do not melt but degrade upon heating above a certain temperature. The melting point of PVA is close to the values reported in the literature. For example, Yang *et al.* (2004) reported a melting endothermic peak at 223^{o}C which is very close to our value (222°C) while Shi *et al.* (2008) demonstrated that pure PVA film exhibits a melting temperature at 230^{o}C. Also Yang *et al.* (2008) reported a melting point of PVA hydrogel at 228.3^{o}C. In addition, smaller peaks of melting transition at different temperatures appeared in the DSC

curves of CS/PVA blend films with various blend ratios from 90/10 to 50/50 ranging from 215 to 217.3°C. This shows that there is a little shift of the endothermic melting transition to lower temperature with increasing chitosan content in the blends. This is because increasing the CS content in the blend reduces the PVA crystallinity, and therefore decreases the melting point of PVA. As a consequence, the melting depression in CS/PVA blends may indicate that the blends are miscible. The decrease in the melting point with increase in CS content in the CS/PVA blend has been reported in the literature (Khoo *et al*, 2003; Lewandowska, 2005; Chen *et al*, 2007; Jia *et al*, 2007).

These observations are in agreement with the results obtained from CS/AG blended films concerning the results obtained from the first run curves of DSC. However, no T_m for the CS/AG blended films could be obtained from the second heating run, which could be due to the fact that polysaccharides degrade but do not melt.

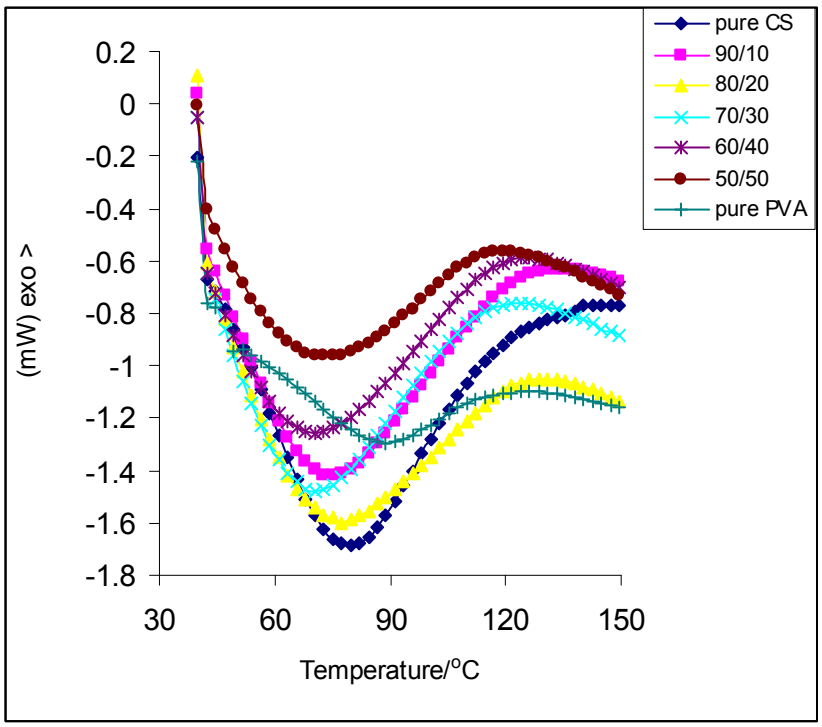

Figure 6.8: The DSC first - run curves of the pure chitosan and pure PVA films together with their blended films.

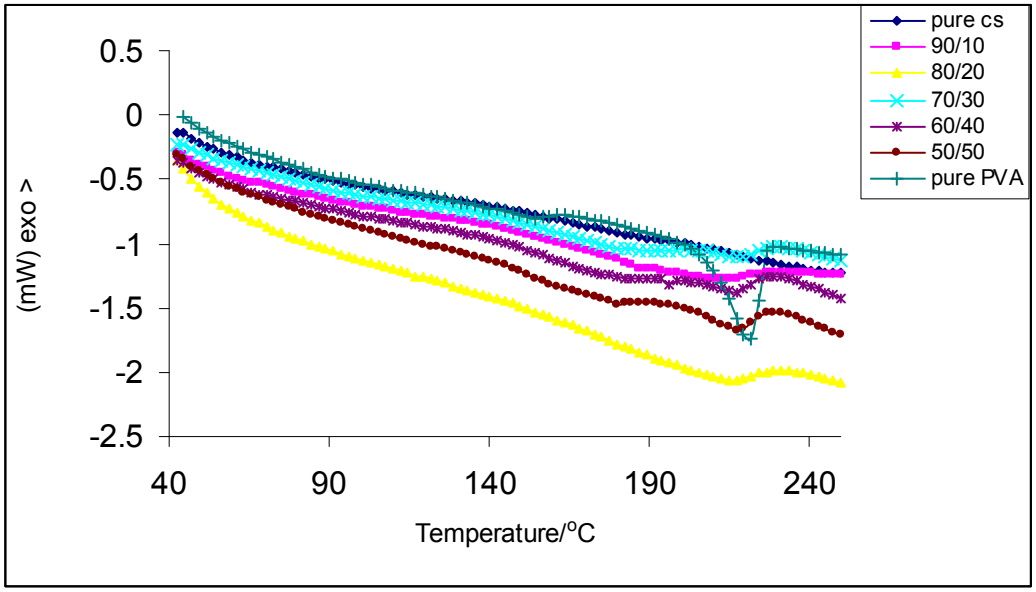

Figure 6.9: The DSC second - run curves of the pure chitosan and pure PVA films together with their blended films.

Table 6.3: The thermal properties of the pure chitosan and pure PVA films together with their blended films with different PVA contents.

Blend composition CS/PVA	100/0	90/10	80/20	70/30	60/40	50/50	0/100
T_D (first run)	79.0	75.0	77.3	70.3	70.3	72.6	89.0
ΔH (J/g) (first run)	227.6	369.4	389.6	326.2	297.1	223.4	ND
T_m (second run)	ND	214.9	214.9	217.3	217.3	217.3	221.9

ND = not detected

6.6 Swelling behavior

Figure 6.10 shows the degree of swelling of pure CS and pure PVA films together with their blended films at various ratios. All the blended films showed much higher degree of swelling comparing with the pure CS film, indicating their higher hydrophilicity. The degree of swelling for the blended films ranging from 1047 to 2117% while the degrees of

swelling for the pure CS and pure PVA films are 89% and 674%, respectively, which indicates that the swelling behavior is greatly influenced by the PVA content in the blend. This is because PVA is a water-soluble polymer and the blending of CS with PVA tends to increase the water uptake due to the increasing of hydrophilic groups (-OH) in the blends. Also, the PVA chains are physically entangled with the CS chains leading to the formation of a hydrogel network (Costa-Júnior *et al*, 2009). It was reported that the blending of CS with the synthetic water-soluble polymer, PVA (Bahrami *et a*l, 2003; Wang and Gunasekaran, 2006) leads to an increase in the water uptake with increasing PVA content. These values are greater than those obtained from CS/AG blended films.

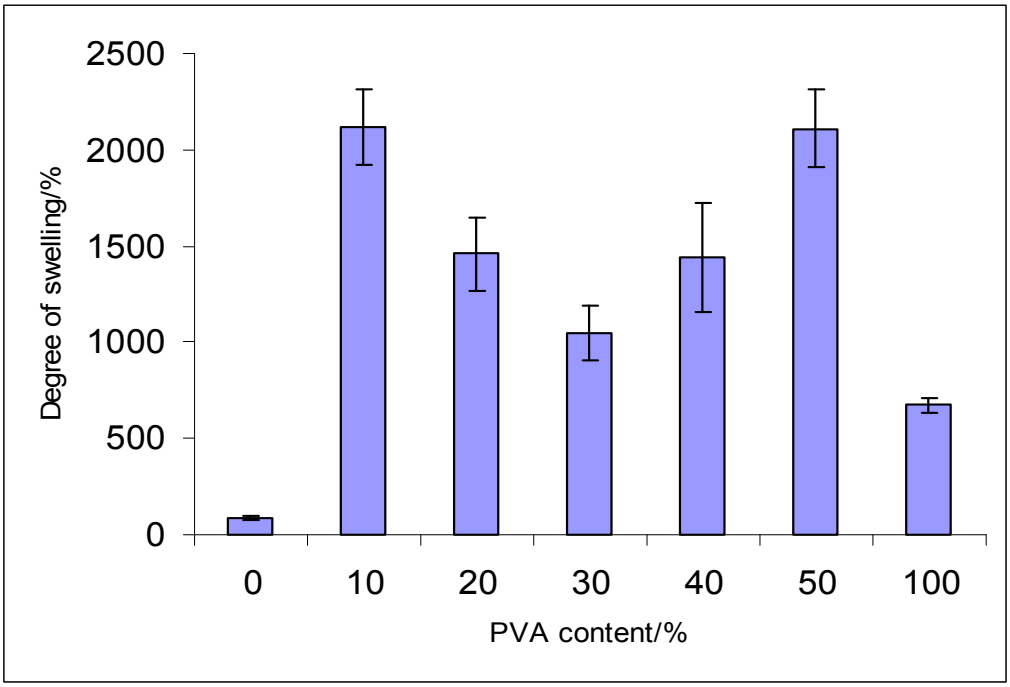

Figure 6.10: The degree of swelling of chitosan/PVA blended films versus the PVA content (0% = pure CS and 100% = pure PVA).

6.7 Contact angle measurements

Figure 6.11 shows the static water contact angle versus the PVA content. A general decrease in the static water contact angle with increasing the PVA content in the blend is observed *i.e.*, there is an increase in the wettability with increasing the PVA concentration in the blend. The contact angle of the blended films ranged from about 70.47± 0.96 to 80.02^{o}± 2.27. In general, the contact angles for all the blended films were less than 90^{o}, indicating good hydrophilicity of the surfaces. These observations are similar to that of the CS/AG blended films.

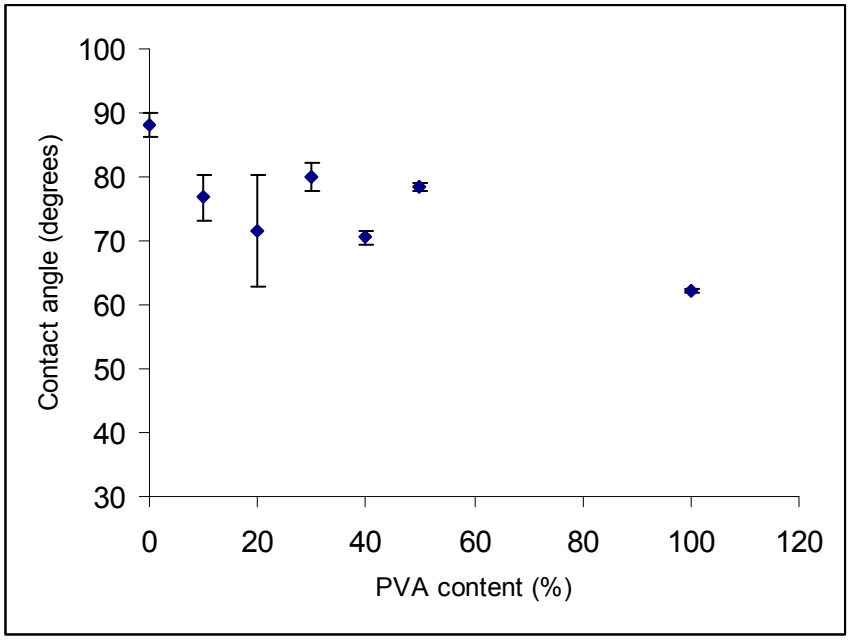

Figure 6.11: The relationship between the water-contact angle and the PVA content for chitosan/PVA blended films having various proportions (0% = pure CS and 100% = pure PVA).

7.0 Chapter 7: Characterizations of chitosan/agar/poly vinyl alcohol (CS/AG/PVA) ternary blended films

7.1 FTIR analysis

The FTIR spectra of pure CS and CS/AG/PVA blended films at different proportions are shown in Figure 7.1. A summary of characteristic bands of pure CS and its blended films with AG and PVA is presented in Table 7.1. As can be seen, the FTIR spectra of the blended films contain the characteristics of the three components. The increase in the AG and PVA concentrations in the blended films caused a little decrease in the intensity of the band arising from the NH bending (amide II) at 1561 cm^{-1} of CS. Also, an increase in the intensity (with little shift to a higher wave number) of CH group at around 2928 cm^{-1} was observed as the AG and PVA contents increase. In addition, the band at about 933cm^{-1}, which is attributed to the 3,6-anhydro-galactose bridges in agar (Chirapart *et al*, 1995), was disappeared in the spectra of the pure CS film and the CS/AG/PVA blended film containing AG and PVA concentrations of 10% each. However, the intensity of this band was increasing for the other ratios as the contents of AG and PVA increased. These observations indicate the existence of compatability among CS, AG and PVA, which is attributed to the formation of intermolecular hydrogen bonds between the amino and hydroxyl groups in CS and the hydroxyl groups in AG and PVA.

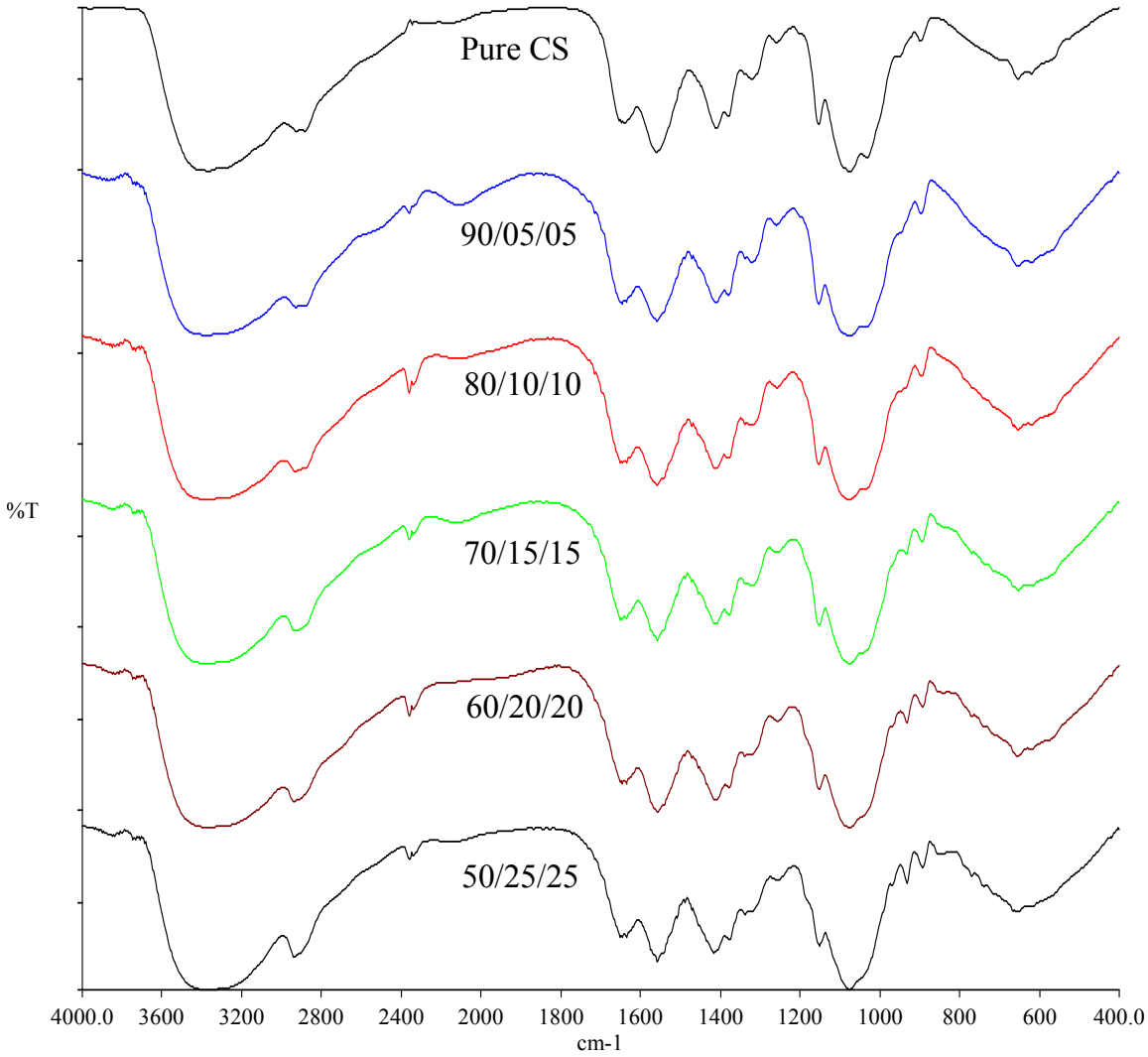

Figure 7.1: Typical FTIR spectra of pure chitosan and CS/AG/PVA blended films having various proportions.

Table 7.1: FTIR characteristic bands (cm^{-1}) of pure CS film and CS/AG/PVA ternary blended films having different proportions

CS/AG/PVA (%)	3,6-anhydro-galactose bridges	C-O	C-O stretch	NH amide II	C=O (amide I)	CH	OH
100/0	-	1033	1077	1561	1647	2928	3368
90/05/05	-	1033	1077	1559	1647	2931	3367
80/10/10	934	1033	1077	1559	1647	2935	3368
70/15/15	934	1033	1077	1559	1647	2936	3368
60/20/20	934	-	1077	1559	1648	2940	3368
50/25/25	933	-	1076	1559	1648	2940	3368

7.2 SEM measurements

The SEM micrographs of the pure CS film and the CS/AG/PVA blended films at different ratios are shown in Figure 7.2. All blended films displayed clear and homogenous surfaces with almost no pores nor interface layer. However, the ratio 50/25/25 showed a little rougher surface indicating more hydrophilic top surface than the other blended films. According to Chen *et al.* (2008b), such a rough surface could be due to the reorientation of polar functional groups toward the top surface of the ternary blended films. Based on these observations it can be concluded that all blended films were compatible at all ratios. Recently, the preparation and characterization of a ternary film of chitosan/poly (vinyl alcohol)/pectin have been reported by Tripathi *et al*. (2010). SEM micrographs showed that the surface of this ternary film is rough and heterogeneous with some immiscibility. Also, Chen *et al*. (2008b) reported the characterization of chitosan/poly (vinyl alcohol)/gelatin ternary blended films. SEM results showed that the top surface of the ternary blend film is hydrophilic with a slight roughness. Similar observations were obtained from CS/AG and CS/PVA blended films in the previous chapters.

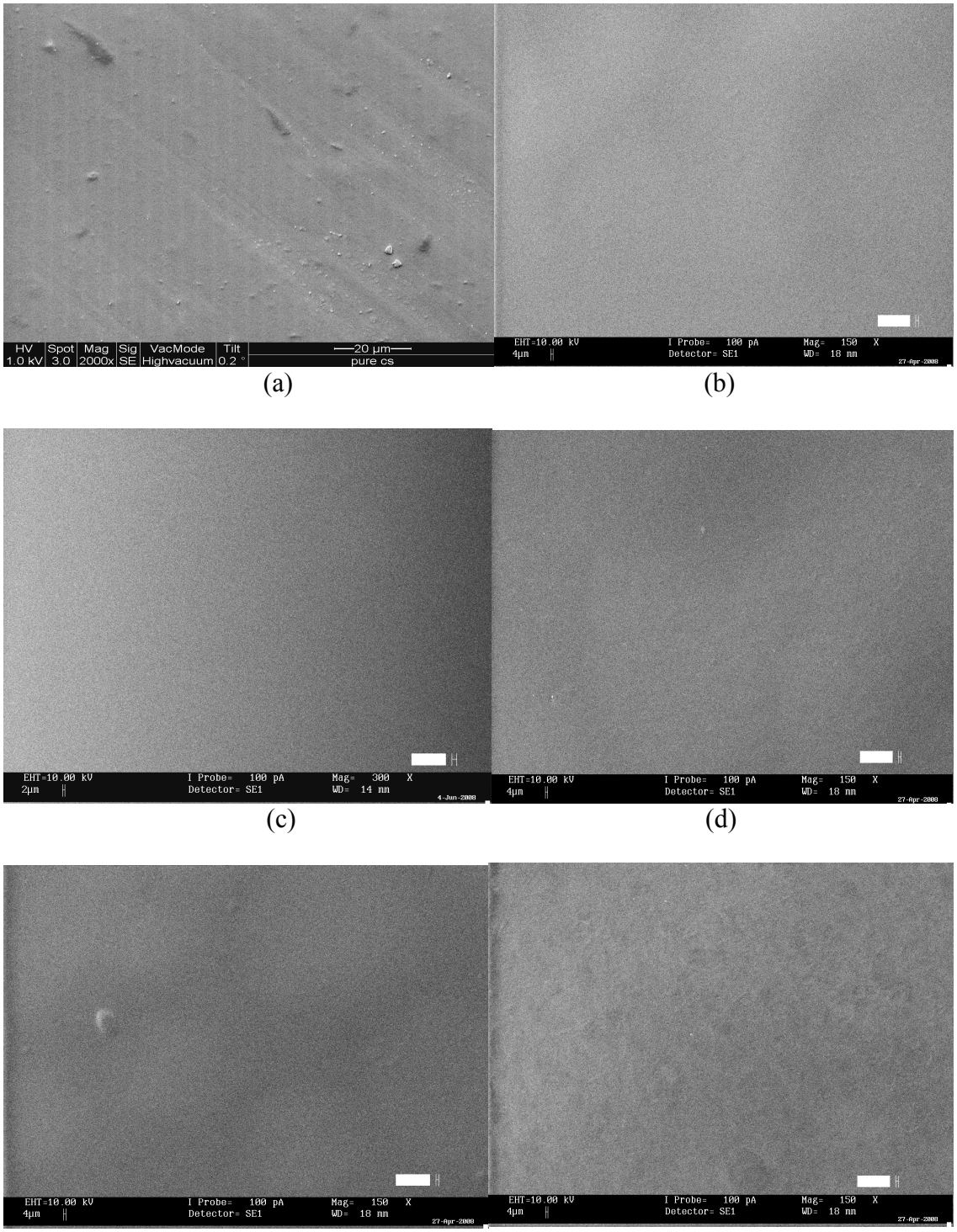

Figure 7.2: The scanning electron micrographs of of: (a) pure CS film together with its blended films at various proportions: (b) 90/05/05, (c) 80/10/10, (d) 70/15/15, (e) 60/20/20 and (f) 50/25/25.

7.3 Tensile strength and elongation % at break

Figures 6.4 and 6.5 show the tensile strength and the elongation % at break of the pure CS film and its ternary blended films with AG and PVA at different proportions. As it can be seen, all the CS/AG/PVA ternary blended films recorded higher values of tensile strength than the pure CS. The tensile strength increased gradually as the amounts of AG and PVA increased until the ratio 60/20/20, which recorded the highest value of TS, *i.e*., 50.8 MPa, then the TS decreased slightly. This improvement in tensile strength could be due to the interaction between –OH and $–NH_2$ groups of CS and –OH groups of AG and PVA. It was also found that the pure CS recorded a higher percentage of elongation than all the ternary blended films. This indicates that the ternary blended films were more brittle than the pure CS film. Similar observations were obtained from CS/PVA blended films unlike CS/AG blended films, which behave differently.

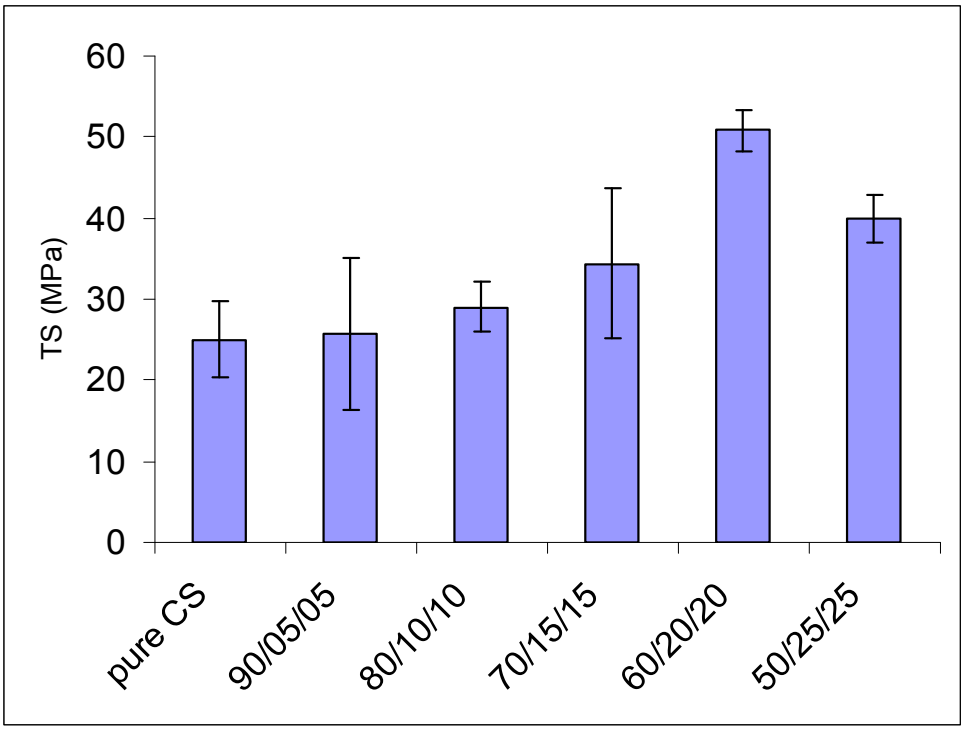

Figure 7.3: Tensile strength of pure chitosan film and its ternary blended films with agar and PVA having various proportions.

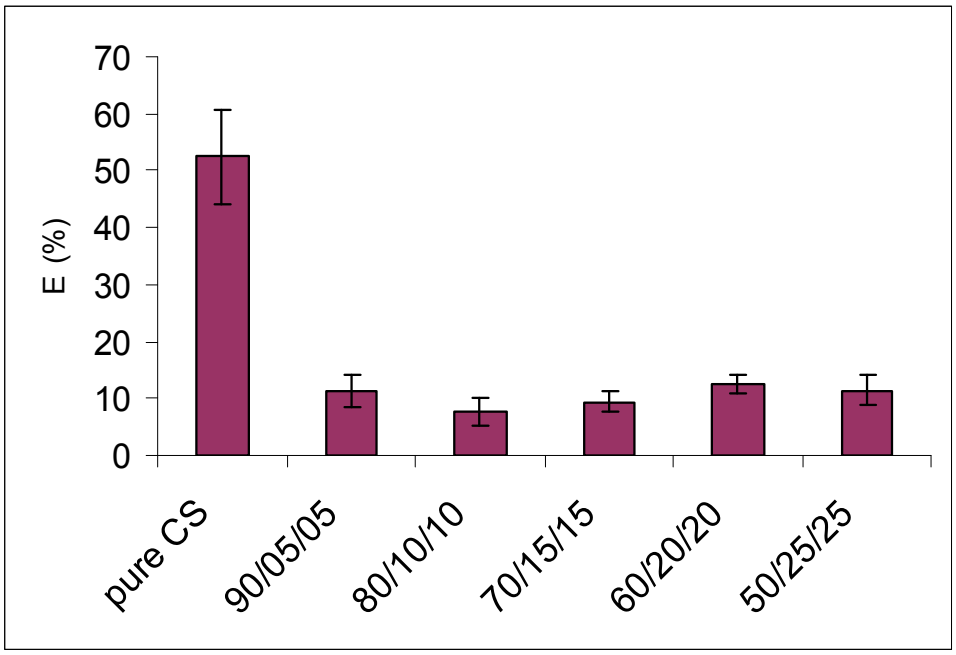

Figure 7.4: Elongation % of pure chitosan film and its ternary blended films with agar and PVA having various proportions.

7.4 TGA measurements

Figure 7.5 shows the TGA curves of the pure CS and CS/AG/PVA ternary blended films at different ratios. The ternary blended films show identical TGA curves (very close degradation behavior) marked by the presence of two weight losses in the temperature range of 40-500^{o}C. The first weight loss, which appears at about 130^{o}C is due to the moisture vaporization. The second weight loss, which starts at about 150^{o}C and indiscriminately continues up to 500^{o}C can be ascribed to the thermal degradation of the main chain scissions of CS, PVA, and AG. It can be also seen that all the blended films have only one onset degradation temperature trend regardless of composition indicating the existence of interactions among the three components in the blends (Wu *et al*, 2008). This behavior can be attributed to the formation of hydrogen bonding among the functional

groups present in the blend components. It can be suggested that the three components involved in the blended films, *i.e.*, CS, AG, and PVA are highly compatible. It is noteworthy mentioning that the blended films did not undergo complete degradations at the end of the heating run due to the formation of complex residues containing C, N and O. These observations show a good agreement with the CS/AG and CS/PVA blended films reported in the previous chapters. The weight loss data obtained from TGA for these blends are summarized in Table 7.2.

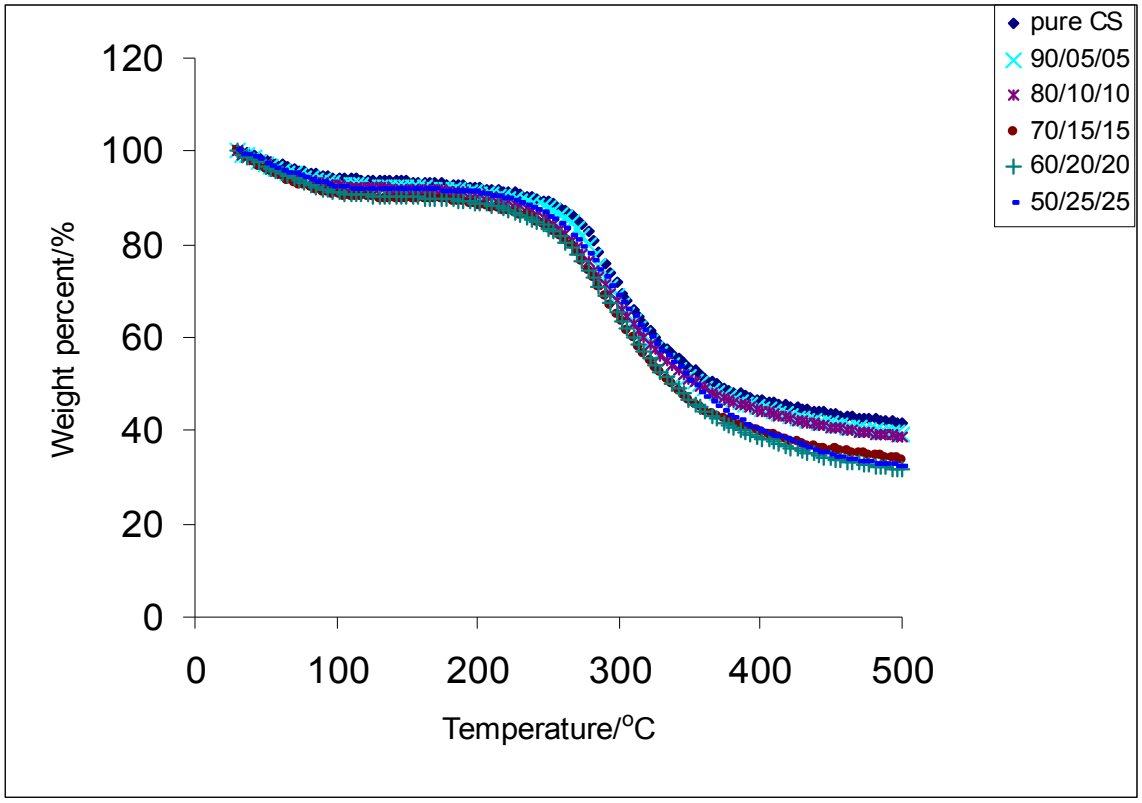

Figure 7.5: TGA curves of pure chitosan film and its ternary blended films with agar and PVA having different proportions.

Table 7.2: The weight loss (%) of the pure chitosan film and its ternary blended films with agar and PVAhaving various proportions at different temperatures.

Temperature (^oC)	Pure CS	90/05/05	80/10/10	70/15/15	60/20/20	50/25/25
100	5.7	7.0	7.5	9.3	8.9	7.7
200	7.9	9.0	9.4	11.5	11.2	9.1
300	30.4	33.1	33.9	36.6	36.4	32.56
400	53.5	55.3	55.7	60.3	61.6	60.2
500	58.3	60.7	61.3	66.0	68.4	67.7

7.5 DSC measurements

Figure 7.6 shows the DSC curve of the first run of CS film and its ternary blended films with AG and PVA having various proportions. The pure CS film shows a broad endothermic peak at about 79^{o}C, which is attributed to the bound water in the film. The blended films exhibited broad endothermic peaks range from about 77.3 to 79.6^{o}C (Table 7.3). Figure 7.6 also shows that there is no much difference in the endothermic peak area of the films, *i.e.,* they don't vary much in their water-holding capacity. Another point to be noted is that all ratios have lower values of T_D than the pure CS except the ratio 70/15/15 which showed higher T_D. This variation on the position could be due to the physical and molecular changes caused by blending of the three polymers.

Figure 7.7 shows the second heating run of the DSC, which was conducted to decrease the water content in the films as it has been mentioned earlier. The first point to note is the absence of the endothermic peak confirming that this peak is attributed to the water content in the samples. Also, smaller peaks of melting transition at different temperatures appeared in the DSC curves of CS/AG/PVA blended films with various blend ratios ranging from 217.3 to 219.6°C as shown in Table 5.7. Based on these observations, it can be concluded

that the interaction among CS, AG and PVA may occur to form films with better thermostability. Similar behavior and results were obtained from the first heating runs of CS/AG blended films and from the first and second runs of CS/PVA blended films.

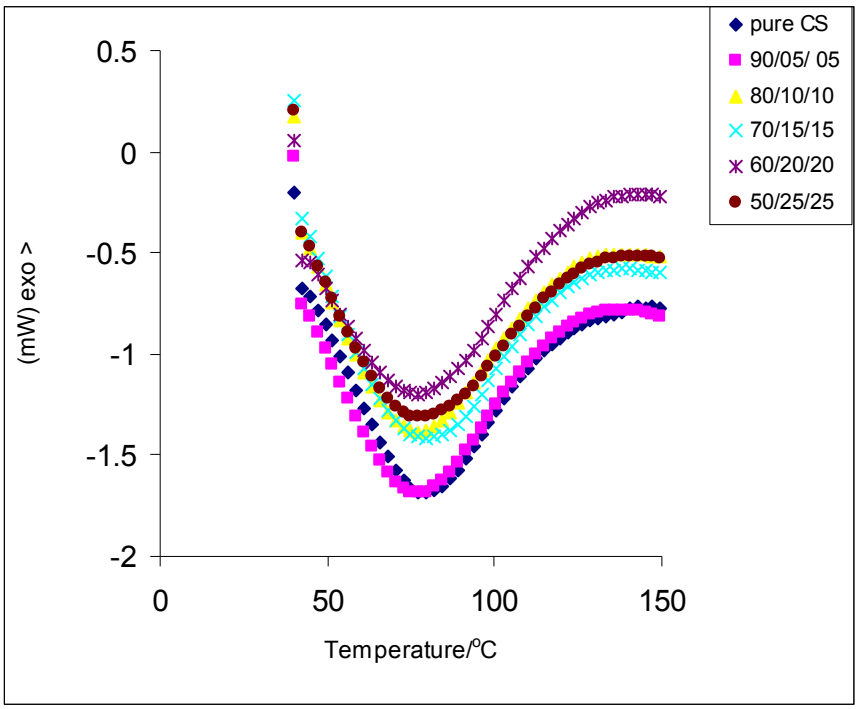

Figure 7.6: The DSC first - run curves of the pure chitosan film and its ternary blended films with agar and PVA having various proportions.

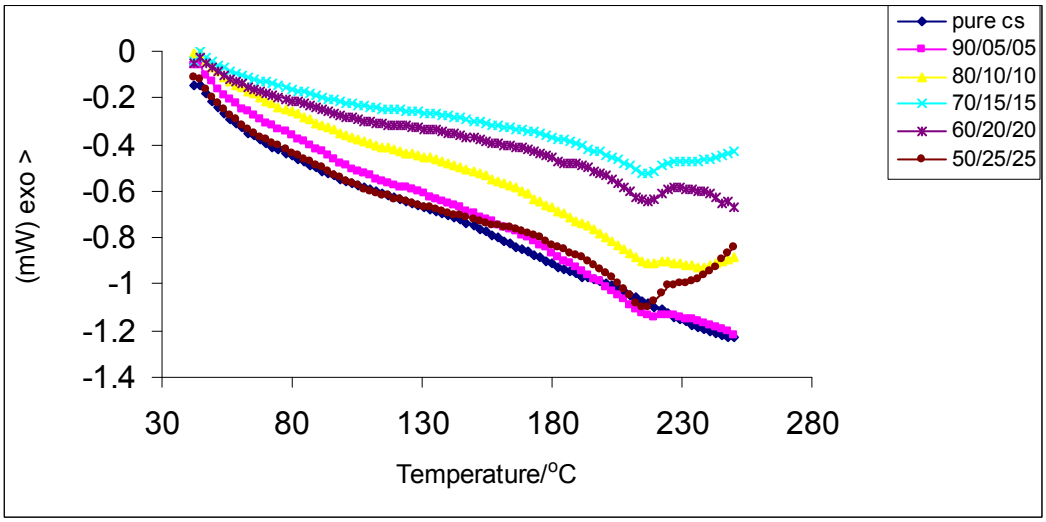

Figure 7.7: The DSC second - run curves of the pure chitosan film and its ternary blended films with agar and PVA having various proportions.

Table 7.3: The thermal properties of the pure chitosan film and its ternary blended films with agar and PVA at different ratios.

Blend composition CS/AG/PVA	100/0/0	90/05/05	80/20/20	70/15/15	60/20/20	50/25/25
T_D (first run)	79.0	77.3	77.3	79.6	77.3	77.3
ΔH (J/g) (first run)	227.6	244.1	255.7	283.9	214.3	246.1
T_m (second run)	ND	219.6	219.6	217.3	217.3	219.6

7.6 Swelling behavior

Figure 7.8 shows the degree of swelling of the pure CS film together with its ternary blended films with AG and PVA at various ratios. All the ternary blended films showed much higher degree of swelling comparing with the pure CS film, indicating their higher hydrophilicity. The degree of swelling for the ternary blended films ranges from 488 to 855%, which indicates that the swelling behavior is influenced by the AG and PVA contents in the blend. This is acceptable since both of AG and PVA contain hydrophilic groups (-OH). These observations are intermediate between those obtained from CS/AG and CS/PVA blended films reported in the previous chapters.

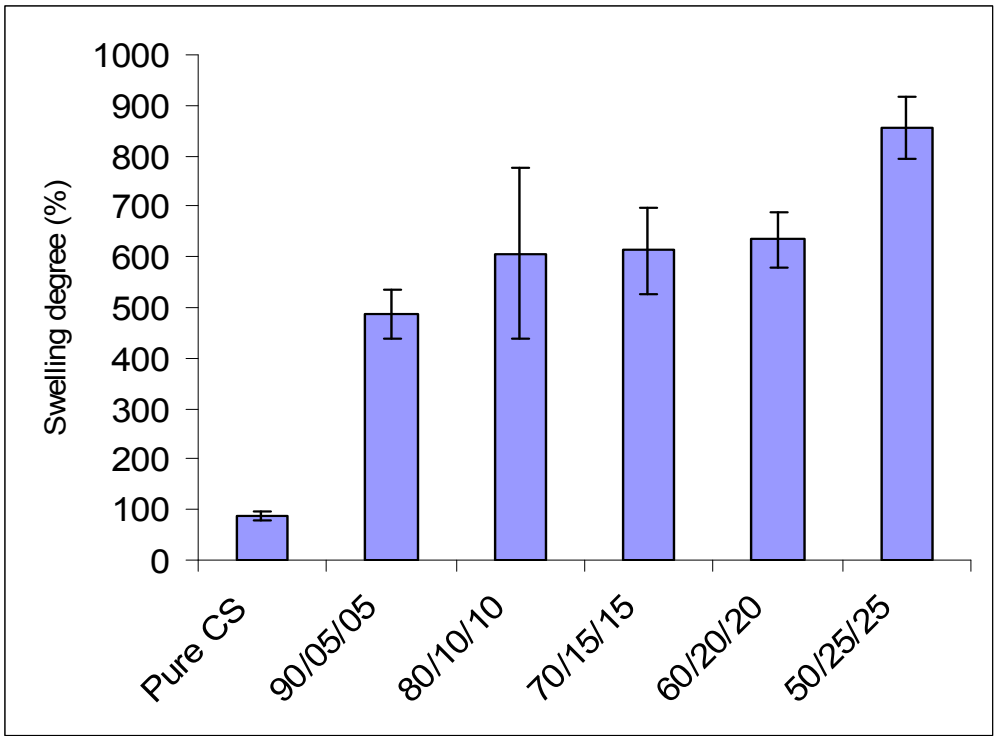

Figure 7.8: The degree of swelling of the pure chitosan film together with its ternary blended films with AG and PVA at various ratios.

7.7 Contact angle measurements (surface hydrophilicity)

Figure 7.9 shows the static water contact angle of the pure CS film and its ternary blened films with AG and PVA at various proportions. There is a general decrease in the static water contact angle with increasing the AG and PVA contents in the blend *i.e.,* an increase in the wettability of the surface of the ternary blended films. This means that the surface became more hydrophilic as the concentrations of AG and PVA increased. The contact angle of the pure CS was 88^{o}±1.91 while the contact angle of the ternary blended films ranged from about 88± 2.15 to 80^{o}± 0.75. The lowest value of contact angle was recorded for the blended film of 50/25/25 indicating best hydrophilicity. This was in agreement with the SEM images since this ratio showed roughest surface among all surfaces investigated.

However, generally, the contact angles for all the blended films were less than 90^{o}, indicating good hydrophilicity of the surfaces. These results are in good agreement with those obtained from CS/AG and CS/PVA blended films in that all the blended films show improvement in the wettability of the surfaces.

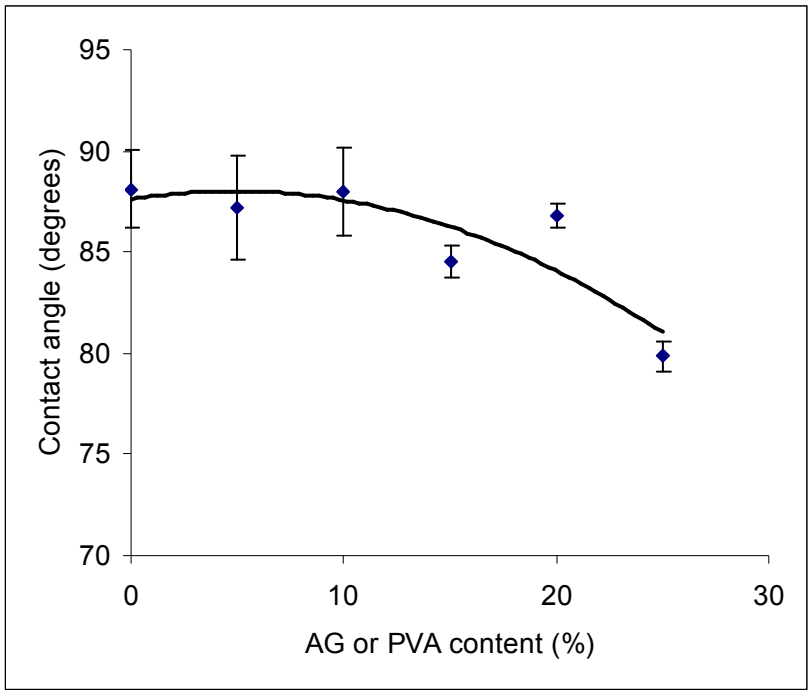

Figure 7.9: The relationship between the water-contact angle and the agar or PVA content for the chitosan/agar/PVA ternary blended films having various ratios (0% = pure CS).

8.0 Chapter 8: Conclusions and recommendation for future work

8.1 Conclusions

A number of aqueous mixture solutions of chitosan/agar (CS/AG), chitosan/ poly vinyl alcohol (CS/PVA) and chitosan/agar/poly vinyl alcohol (CS/AG/PVA) at different proportions were prepared. The effects of temperature, shear rate, shearing time and storage time on the rheological properties, *i.e.,* the apparent viscosity and the shear stress as a function of shear rate were investigated for these systems. Also, blended films of chitosan/agar chitosan/PVA and chitosan/agar/PVA were prepared at various proportions. The chemical structure and the morphology of the obtained blended films were investigated using Fourier transform infrared (FTIR) and field emission scanning electron microscope (FESEM). The thermal stability of the blended films was studied using thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC). In addition, the swelling, contact angle and mechanical properties of the blended films were investigated. Based on the present study, various conclusions could be drawn as stated in the next paragraphs.

This study has shown that the pseudoplastic non-Newtonian behavior was observed only at high concentrations of AG for CS/AG and CS/AG/PVA blended solutions at the range of temperature studied which suggests the existence of interaction among the blend solutions' components. However, all the CS/PVA blended solutions displayed a Newtonian behavior. In addition, the relationship between the apparent viscosity and the temperature could be described with the Arrhenius equation for all blended solutions. This study has also shown that curves of the apparent viscosity of CS/AG blended solutions exhibited similar behavior at all shearing times of 15-75 s. However, the solution containing ratio of 50% of agar exhibited a decrease in viscosity and shear stress with increasing shear rate. In addition, all

CS/PVA blended solutions showed similar behavior at all shearing times of 15-75 s. Furthermore, the curves of the apparent viscosity of the CS/AG/PVA ternary system showed almost no change in the apparent viscosity at all shearing times applied for lower concentrations than 15% of each of AG and PVA. However, a rheopectic behavior was observed at higher concentrations of the two polymers. When the storage period was extended to three weeks, all the CS/AG blended solutions as well as the CS/AG/PVA ternary blended solutions recorded remakably higher increase in the viscosity as the time prolonged compared to chitosan solutions, especially, in the first week. However, the CS/PVA blends recorded a decrease in the viscosity with time in general compared to the pure chitosan solution. Based on the above findings, it can be concluded that the interaction between the three components has occurred except in the CS/PVA blends in which it was difficult to observe any interaction by rheological measurement.

Blends of CS and AG in film forms were prepared at various proportions in the range of 90/10-50/50 (v:v). Investigations of the properties of the obtained blend films of chitosan/agar revealed that there is a high compatibility between CS and AG as confirmed by the results of FTIR, TGA and FESEM results. Introducing agar was found to decrease the tensile strength and the elongation % of the CS/AG blended films and such effect is a function of AG content. On the other hand, introducing AG to the blended films was found to enhance the thermal stability of some blended films under the influence of strong intermolecular hydrogen bonding existing between the amino groups of CS and the hydroxyl groups of AG. Studying the swelling behavior of the blended films showed that the water uptake was increased for all blend films compared to the pure CS film, indicating an enhancement in the hydrophilicity. Static water contact angle measurements confirmed

the increasing affinity of the blended films towards water suggesting that blending of AG with CS improves the wettability of the obtained films.

In addition, blended films of CS and PVA were prepared at various proportions in the range of 90/10-50/50 (v:v). Investigation of the properties of the obtained blends displayed some interaction between CS and PVA as revealed by the results of FTIR, FESEM and TGA due to the intermolecular hydrogen bonding existing between the amino groups of CS and the hydroxyl groups of PVA. The blended films also showed an increase in the tensile strength and a decrease in elongation at break with increasing PVA content. Studying the swelling behavior showed much higher water uptake for all blended films compared to that of the pure CS film, indicating an enhancement in the hydrophilicity. The water contact angle measurements confirmed the increase in the hydrophilicity of the blended films

Furthermore, blended films of CS/AG/PVA were prepared at various proportions. Investigations of the obtained blended films with FTIR, FESEM and thermal stability studies revealed that miscibility between CS, AG and PVA was achieved together with a relatively smooth and homogeneous surface morphology as depicted from FESEM images. The mechanical properties of the blended films showed an increase in the tensile strength and a decrease in the elongation % at break with the increase in the content of the AG and PVA. The water uptake of all blended film was found to increase drastically compared to the pure CS film, indicating a sharp rise in the hydrophilicity upon addition of both of AG and PVA. These observations indicate the existence of strong interamolecular hydrogen bonds between the amino groups of CS and the hydroxyl groups of AG and PVA. The water contact angle measurements confirmed the increase in the hydrophilicity of the ternary blended films, which reached its maximum value at the composition ratio 50/25/25

as indicated by its minimum value of water contact angle (80^{o}). This suggests that the blending of CS with AG and PVA improves the surface wettability of the blended films.

8.2 Recommendation for future work

1. Since the blended films obtained in this work showed some improvements in their physical properties which could promotes their use in various applications, it is recommended to test the oxygen and water vapor permeability to explore their suitability for food packaging application or packaging for medical supplies.

2. Crosslinking of these blended films is highly recommended to further improve, for example the mechanical properties of the blended films, which is important criteria to be matched with the requirement for specific applications. Cytotoxic synthetic crosslinking reagents, such as glutaraldehyde and tripolyphosphate, should be avoided, especially, if the pharmaceutical and biomedical applications are considered. Instead, naturally occurring cross-linking agents, such as genipin, might be attempted. If crosslinking is considred the level of crosslinking has to be optimized to avoid underming of other properties such as swelling.

3. Since these blended films have shown high degree of swelling and known to be biodegradable and non-toxic, this may indicate that they could be used for biomedical applications such as wound healing upon loading with healing agent.

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Appendix

a. Relationship between the R^2 and the blend concentration.

Blend ratio (CS/AG)	Equation	R^2
100/0	Y = - 0.2296x + 22.591	0.9821
90/10	Y = - 0.1248x + 13.548	0.9869
80/20	Y = - 0.1128x + 12.888	0.9796
70/30	Y = - 0.1144x + 12.764	0.9567
60/40	Y = - 0.3766x + 29.076	0.9934
50/50	Y = - 0.6552x + 62.342	0.9972
0/100	Y = - 4.0746x + 288.86	0.9398

Blend ratio (CS/PVA)	Equation	R^2
100/0	Y = - 0.2296x + 22.591	0.9821
90/10	Y = - 0.2016x + 19.772	0.9966
80/20	Y = - 0.1128x + 11.206	0.9973
70/30	Y = - 0.1128x + 11.206	0.9973
60/40	Y = - 0.1200x + 11.817	0.9615
50/50	Y = - 0.0864x + 9.142	0.9818
0/100	Y = - 0.0312x + 3.1313	0.8824

Blend ratio CS/AG/PVA	Equation	R^2
100/0/0	Y = - 0.2296x + 22.591	0.9821
90/05/05	Y = - 0.2012x + 19.752	0.9964
80/10/10	Y = - 0.1464x + 17.724	0.9553
70/15/15	Y = - 0.1318x + 18.168	0.9912
60/20/20	Y = - 0.3532x + 35.557	0.9873
50/25/25	Y = - 0.528x + 45.715	0.8648

b. The Arrhenius Equation

The Arrhenius Equation can be written as:

$$\eta = A \cdot e^{-Ea/RT}$$

where A is a constant related to molecular motion, E_a is the activation energy for viscous flow at a constant shear rate, R is the gas constant and T is the temperature (in kelvin).

Graphical determineation of the activation energy

$$\ln(\eta) = \ln(A \cdot e^{-Ea/RT}) \qquad \text{Taking the natural log}$$

$$\ln(\eta) = \ln(A) + \ln(e^{-Ea/RT}) \qquad \text{Expand expression}$$

$$\ln(\eta) = \ln(A) - (1/R.T).\ E_a \qquad \text{Expand expression}$$

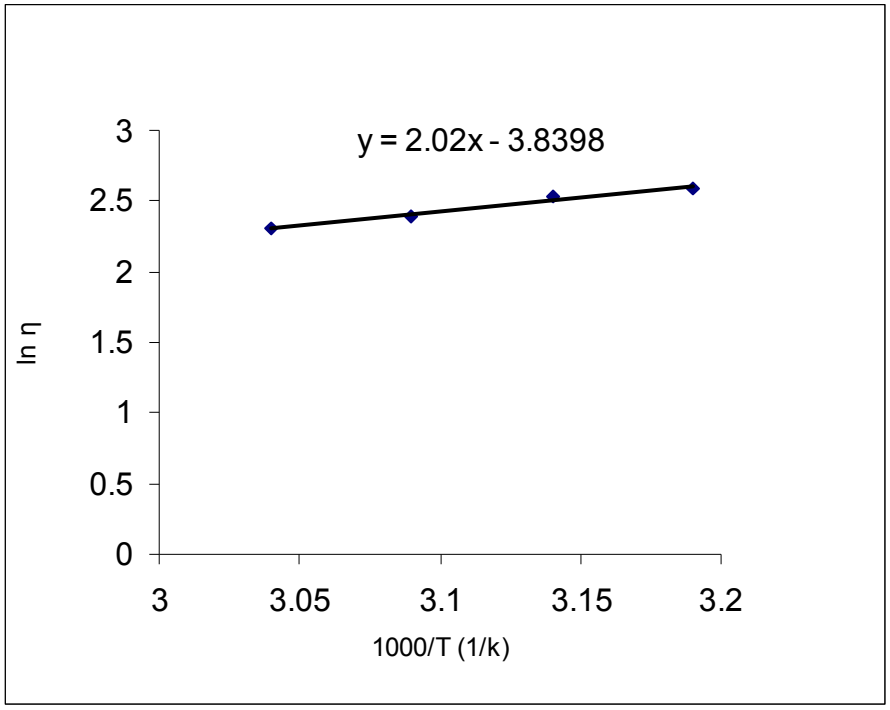
$$\ln(\eta) = \ln(A) - (E_a/R).(1/T) \iff \ln(\eta) = -(E_a/R).(1/T) + \ln(A)$$

This has the form y = mx + b

where 1/T is the x axis , ln(η) is the y axis, ln(A) is the y intercept and (-Ea/R) is the slope.

Sample calculation of the activation energy

Plotting ln(η) against 1/T gives a straight line with a slope of – Ea/R as shown in the following Figure (for pure CS).



The best fitted line showed an equation of y = 2.02x -3.8398 where

$-(E_a/R) = 2.020$

Therefore

$E_a = 16.79$ kJ mol^{-1}

List of publications

Publications from this work

1. **Esam A. El-hefian** and Abdul H. Yahaya. (2010). Rheological study of chitosan and its blends: An overview. *Maejo International Journal of Science and technology*. **4**: 210-220. *(ISI and SCOPUS -Cited Publication)*

2. **Esam A. El-hefian**, Mohamed Mahmoud Nasef, Abdul Hamid Yahaya and Rashid Atta Khan. (2010). Preparation and characterization of chitosan/agar blends: Rheological and thermal studies. *Journal of the Chilean Chemical Society*. **55**: 130-136. *(ISI-Cited Publication)*

3. **Esam A. El-hefian**, Elham S. Elgannoudi, A. Mainal and A. H. Yahaya. (2010). Characterization of chitosan in acetic acid: Rheological and thermal studies. *Turkish Journal of Chemistry*. **34**: 47-56. *(ISI-Cited Publication)*

4. **Esam A. El-hefian**, Mohamed Mahmoud Nasef and Abdul Hamid Yahaya. (2010). Rheological and morphological studies of chitosan/agar/poly (vinyl alcohol) blends. *Journal of Applied Sciences Research.* **6**: 460-468. *(SCOPUS-Cited Publication)*

5. **Esam A. El-hefian**, Mohamed Mahmoud Nasef and Abdul Hamid Yahaya. (2010). The Preparation and Characterization of Chitosan/Poly (Vinyl Alcohol) Blended Films. *E-Journal of Chemistry*. **7**: 1212-1219. *(ISI and SCOPUS-Cited Publication)*

6. **Esam A. El-hefian** and Abdul H. Yahaya. (2010). The effects of temperature, shearing time and rate of shear on the apparent viscosity of chitosan/agar mixture solutions. *Maejo International Journal of Science and technology.* **4**: 261-267. *(ISI and SCOPUS-Cited Publication)*

7. Esam A. El-hefian, Rashid Atta Khan and Abdul Hamid Yahaya, Characterization of aqueous solutions of mixture of chitosan and agar. *Journal of the Chemical society of Pakistan*. Accepted manuscript. *(ISI-Cited Publication)*

8. **Esam A. El-hefian**, Mohamed Mahmoud Nasef and Abdul Hamid Yahaya. Preparation and characterization of chitosan/poly vinyl alcohol blends: Rheological study. *E-Journal of Chemistry*. Accepted manuscript.

9. **Esam A. El-hefian**, Mohamed Mahmoud Nasef and Abdul Hamid Yahaya. The preparation and characterization of chitosan/poly (Vinyl Alcohol) blended films: Mechanical, thermal and surface investigations. *E-Journal of Chemistry*. Accepted manuscript. *(ISI and SCOPUS-Cited Publication)*

10. **Esam A. El-hefian**, Mohamed Mahmoud Nasef and Abdul H. Yahaya. (2010). Mechanical, thermal and surface investigations of chitosan/agar/PVA ternary blended films. *Maejo International Journal of Science and technology.* Submitted.

11. **Esam A. El-hefian,** Mohamed Mahmoud Nasef and Abdul H. Yahaya. (2010). The preparation and characterization of chitosan/agar blend film. *International Journal of Biological Macromolecules*. Submitted.

12. **Esam A. El-hefian,** Mohamed Mahmoud Nasef and Abdul H. Yahaya. (2010). The preparation and characterization of chitosan/agar/poly (vinyl alcohol) ternary blended films. *Journal of the* ***Serbian Chemical*** *Society*. Submitted.

Papers in progress

1. Chitosan-based polymer blends: current status and applications: A review.

Conferences from this work

1. **Esam A. El-hefian**, Elham. S. Elgannoudi, Azizah Mainal and A. H. Yahaya: *Rheology of chitosan solutions in acetic acid*. International Graduate Congress 2007: 3rd Mathematics & Physical Science Graduate Congress (MPSGC).Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia. (12-14 December 2007).

2. **Esam A. El-hefian**, R. Yahya, Mohamed Mahmoud Nasef, Rashid Atta Khan and A. H. Yahaya: *Characterization of aqueous solutions of mixture of chitosan and agar*. 2nd Penang International Conference For Young Chemists (ICYC). University Sains Malaysia, Penang, Malaysia. (18-20 June 2008).

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